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(54) Title: METHOD FOR TYPING AND DETECTING HBV

(57) Abstract

The present invention relates to a method for detection and/or genetic analysis of HBV in a biological sample, comprising hybridizing the polynucleic acids of the sample with a combination of at least two nucleotide probes, with said combination hybridizing specifically to a mutant target sequence chosen from the HBV RT pol gene region and/or to a mutant target sequence chosen from the HBV preCore region and/or to a mutant target sequence chosen from the HBsAg region of HBV and/or to a HBV genotype-specific target sequence, with said target sequences being chosen from Figure 1, and with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of the sample under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U; and detecting the hybrids formed; and inferring the HBV genotype and/or mutants present in said sample from the differential hybridization signal(s) obtained. The invention further relates to sets of nucleotide probes and possibly primers useful in said methods as well as to their use in a method for typing and/or detecting HBV and to assay kits using the same.

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Method for typing and detecting HBV

The present invention relates to the field of Hepatitis B virus (HBV) diagnosis. More particularly, the present invention relates to the field of HBV genotyping and/or determination of the presence of HBV mutants in test samples.

The present invention relates particularly to a method for the rapid and reliable detection of HBV mutants and/or genotypes occuring in a test sample using specific sets of probes optimized to function together in a reverse-hybridisation assay.

Hepatitis B virus is a small enveloped DNA virus of approximately 3200 bp long. Historically it has been characterized on the basis of immunological reaction of the HBsAg with sets of monoclonal antibodies. Isolates were described as *a,* indicating the common determinant for all different subtypes, followed by subtype-specific combinations: *dw, dr, yw,* or *yr.* The latter are mutually exlusive pairs of determinants, covering the HBsAg amino acids 122 (*d*=lys, *y*=arg) and 160 (*w*=lys, *r*=arg). Several subdeterminants for *w* exist and can be ascribed to the appeareance of certain amino acid variants at codon 127. More recently, a genetic classification has been proposed, based on molecular analysis of the virus. This kind of analysis showed that in total six different genotypes exist, indicated from A to F, with a maximum genetic divergence of 8% when comparing complete genomes (reviewed by Magnius and Norder, 1995).

The genetic variability of HBV might be clinically important. Indeed, the genome variability might include some mechanisms by which HBV avoids immune clearance, and hence induces chronic infection. An important protein marker in inducing immune tolerance, virus elimination, and chronic infection, is HBeAg. The expression of this protein is strictly controled both at the transcriptional and translational level (Li et al., 1993; Okamoto et al., 1990; Yuan et al., 1995; Sato et al., 1995) . Therefore, in the natural course of HBV infection, a well characterized stage of the disease is indicated as HBe-negative chronic hepatitis B (reviewed by Hadziyannis S.J., 1995). This phase is mostly due to the appear ance of preCore translational stop codon mutations. The overal genetic

variability determines the frequency and physical location on the viral genome where these translational stop-codon mutations appear. The transcriptional regulation was proposed to be the mechanism for genotype A (and possibly also F), whereas the translational control was more likely to be found in the other genotypes (Li et al.; 1993; Sato et al., 1995). Contradictory to the translational regulation, it was shown that the transcriptional regulation was unable to block the HBeAg expression completely and was therefore proposed to categorize the phenotype of this mutant as HBe-suppressed, rather than as HBe-negative (Takahashi et al., 1995). In any case, these preCore mutants would lead to a destruction of the pre-existing balance between HBeAg in circulation and the HBc-derived peptides presented by class I HLA molecules on the surface of infected hepatocytes, thereby diminishing the supressive effect of HBeAg on T cells, finally resulting in partial liberation of core-specific CTLs and leading to apoptosis of the infected hepatocytes. In general, after the emergence of the HBe-minus variants, the course of the viral infection is characterized by the progression of chronic hepatitis, which may lead to the development of cirrhosis and hepatocellular carcinoma (Hadziyannis, 1995).

Another issue for which the genetic variability or genotyping of the virus might be of relevance is in the development of vaccines where the response may be mediated by the virus type. Protection against HBV infection of all subtypes is conferred by antibodies to the common 'a' determinant of the HB surface antigen (HBsAg). It has been shown that this 'a' determinant presents a number of epitopes, and that its tertiary structure is most important for its antigenicity. The most important region lies between amino acid 124 and 147, but can be extended from amino acid 114 to 150. An adequate anti-HBs response, built up after vaccination, is in principle fully protective. Infection with a HBV strain harboring mutations in the 'a' determinant region might result in vaccine failure, because the vaccine-induced humoral immune response does not recognize the mutant HBsAg. The most common vaccine-associated escape mutants are the substitutions of a glycine at position 145 to an arginine (G145R), K141E, and T126N. But a 2-aa insertion between aa position 122 and 123, and 8-aa insertion between aa 123

and 124 have also been found (Carman et al., 1990, 1995; Crawford, 1990; Waters et al., 1992).

Lamivudine is a (-) enantiomer of 3' thiacytidine, a 2'3'-dideoxynucleoside analogue, and is known to be a potent inhibitor of HBV replication through inhibition of the reverse transcriptase (RT) activity of the HBV polymerase. Lamivudine treatment can result in histological improvements in chronic hepatitis patients, and when given pre- and post-liver transplantation, it can prevent graft reinfection (Honkoop et al., 1995; Naoumov et al., 1995). However, after treatment, a hepatitis flare-up can be observed in most patients, with ALT elevations and HBV DNA that becomes detectable again. This HBV DNA rebound is associated with a new quasi species equilibrium. In a few cases, virus breakthrough during therapy was observed, due to the selection of lamivudine resistent HBV strains. The exact nature of this breakthrough has been ascribed to the accumulation of mutations in the RT part of the Polymerase. A similar mechanism in the HIV RT polymerase has been found, where upon lamivudine treatment, mutations accumulate in the YMDD motif (Gao et al., 1993). This YMDD motif is also present in the RT part of the HBV polymerase, and lamivudine-selected mutations in HBV have been found in this region (Tipples et al., 1996), as well as in other regions of the RT part of the polymerase (Ling et al., 1996). Penciclovir is another drug that has been shown to inhibit the reverse transcriptase activity of the HBV polymerase (Shaw et al., 1996), and mutations in the HBV polymerase may also be detected upon treatment with this drug.

From all this it can be concluded that the information on the following issues is essential for proper *in vitro* diagnosis, monitoring and follow-up of HBV infections:

- genotype;
- preCore mutations;
- vaccine escap mutations;
- RT gene mutations selected by treatment with drugs such as lamivudune and penciclovir.

To obtain all this information using existing technologies is complicat d, time-

consuming, and requires highly-skilled and experienced personel.

It is thus an aim of the present invention to develop a rapid and reliable detection method for determination of the presence or absence of one or more HBV genotypes possibly present in a biological sample.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of the presence or absence of one or more variations in the HBV preS1 region and/or in the HBsAg region representing one or more HBV genotypes possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of the presence or absence of one or more HBV mutants possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of one or more mutations in the preCore region of HBV possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of one or more mutations in the HBsAg region of HBV possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of one or more mutations in the polymerase (pol) gene region of HBV possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for the simultaneous determination of one or several HBV genotypes in combination with one or several HBV mutants possibly present in a biological sample in one single experiment.

It is also an aim of the present invention to provide a genotyping assay or method which allows to infer the nucleotide sequ nce at codons of interest and/or the HBV mutants of interest, and/or inf r the HBV genotype possibly present in a

biological sample.

Even more particularly it is also an aim of the present invention to provide a genotyping assay allowing the detection of the different HBV mutants and genotypes in one single experimental setup.

It is another aim of the present invention to select particular probes able to discriminate one or more HBV mutations in one of the above mentioned regions of the HBV genome and/or able to discriminate one or more HBV genotypes.

It is more particularly an aim of the present invention to select particular probes able to discriminate wild-type HBV from mutant HBV sequences.

It is also an aim of the present invention to select particular probes able to discriminate wild-type and polymorphic variants of HBV from mutant HBV sequences.

It is also an aim of the present invention to select particular probes able to discriminate HBV genotype sequences.

It is moreover an aim of the present invention to combine a set of selected probes able to genotype HBV and/or discriminate different HBV mutants possibly present in a biological sample, whereby all probes can be used under the same hybridisation and wash conditions.

It is also an aim of the present invention to select primers enabling the amplification of the gene fragment(s) determining the HBV genomic mutations or variations of interest as discussed above.

The present invention also aims at diagnostic kits comprising said probes useful for developing such a genotyping assay and/or assays for detecting, monitoring or following-up HBV infection and/or assays for detecting HBV mutations.

All the aims of the present invention have been met by the following specific embodiments.

As a solution to the above-mentioned problem that it is essential for proper diagnosis, monitoring and follow-up of HBV infection to have information on the genotype of HBV present, the present invention provides an elegant way to tackle

problems of such complexity which involves residing to a reverse hybridization approach (particularly on Line Probe Assays strips, as described by Stuyver et al., 1993). Using this technology it is possible to conveniently obtain all essential data in one test run. To achieve this goal, a set of probes needs to be designed and assembled which can detect all relevant polymorphisms in the HBV gene regions of interest.

The present invention thus particularly relates to a method for determining the presence or absence of one or more HBV genotypes in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two nucleotide probes hybridizing specifically to a HBV genotype specific target sequence chosen from Figure 1; with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV genotype present in said sample from the differential hybridization signal(s) obtained in step (iv).

The genotype specific target sequences can be any nucleotide variation appearing upon alignment of the different HBV genomes that permits classification of a certain HBV isolate as a certain genotype (see Figure 1).

The expr ssion "relevant part of a suitable HBV gene" refers to the part of the HBV gene encompassing the HBV genotype specific target sequ nce chosen from Figure 1 to be detected.

According to a preferred embodiment of the present invention, step (iii) is performed using a set of at least 2, preferably at least 3, more preferably at least 4 and most preferably at least 5 probes all meticulously designed such that they show the desired hybridization results, when used in a reverse hybridisation assay format, more particularly under the same hybridization and wash conditions implying that each of said probes is able to form a complex upon hybridisation with its target sequence present in the polynucleic acids of the sample as obtained after step (i) or (ii).

The numbering of the HBV gene encoded amino acids and nucleotides is as generally accepted in literature.

More particularly, the present invention relates to a set of at least 2 probes allowing the detection of a genotype specific variation, possibly also including one or more probes allowing the detection of a wild-type sequence, a polymorphic or a mutated sequence at any one of the nucleotide positions showing a sequence diversity upon alignment of all known or yet to be discovered HBV sequences as represented in Figure 1 for all complete HBV genomes found in the EMBL/NCBI/DDBJ/Genbank.

The sets of probes according to the present invention have as a common characteristic that all the probes in said set are designed so that they can be used together in a reverse-hybridization assay, more particularly under similar or identical hybridization and wash conditions as indicated above and below.

Selected sets of probes according to the present invention include probes which allow to differentiate any of the HBV genotype specific nucleotide changes as represented in Figure 1, preferably in the preS1 or HBsAg region of HBV. Said probes being characterized in that they can function in a method as set out above.

In order to solve the above-mentioned problem of obtaining information on the possible presence of HBV mutants in a given sample, the present invention provides an elegant way to tackle this problem which involves residing to a reverse hybridisation approach (particularly on Line Probe Assays strips, as described by Stuyver et al., 1993). Using this technology it is possible to conveniently obtain all essential data in one test run. To achieve this goal, a set of probes needs to be

designed and assembled which can detect all relevant mutations and possibly also wild-type sequences or polymorphisms in the HBV gene regions of interest.

Another particularly preferred embodiment of the present invention thus is a method for determining the presence or absence of one or more HBV mutants in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two nucleotide probes hybridizing specifically to a HBV mutant target sequence chosen from Figure 1, with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U and with said set or probes possibly also comprising one or more wild-type HBV probes corresponding with the respective mutated HBV target sequence;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV mutant(s) present in said sample from the differential hybridization signal(s) obtained in step (iv).

It is to be understood that the term "mutant target sequence" not only covers the sequence containing a mutation, but also the corresponding wild-type sequence. The HBV mutant target sequence according to the present invention can be any sequence including a HBV mutated codon known in the art or yet to be discovered. Particularly preferred HBV mutant target regions are set out below.

In order to solve the problem as referred to above of obtaining information on the essential issues for proper diagnosis of HBV (namely genotype and different mutations particularly mutations in the preCore region, vaccine escape mutations and RT gene mutations selected by treatment with drugs such as lamivudine and

penciclovir), the present invention provides a particularly elegant way to obtain such complex information.

Moreover, careful analysis of the data obtained by the present inventors clearly revealed that combining the information concerning the preCore and escape mutants with data on the genotype is essential to allow adequate interpretation of the results. Hence it is highly advantageous to be able to produce all relevant data simultaneously.

In this method for diagnosing HBV mutants, preferably in combination with HBV genotyping, a set of probes selected as defined above may be used, wherein said set of probes is characterized as being chosen such that for a given HBV mutation, the following probes are included in said set :

-at least one probe for detecting the presence of the mutated nucleotide(s) at said position;

-at least one probe for detecting the presence of the wild-type nucleotide(s) at said position;

-possibly also (an) additional probe(s) for detecting wild-type polymorphisms at positions surrounding the mutation position.

Inclusion of the latter two types of probes greatly contributes to increasing the sensitivity of said assays as demonstrated in the examples section.

Selected sets of probes according to the present invention include at least one probe, preferably at least two probes, characterizing the presence of a HBV mutation at nucleotide positions chosen from the preCore region of HBV, more particularly from the following list of codons susceptible to mutations in the HBV preCore region, such as codon 15 in genotype A, and for all genotypes: codon 28, codon 29, and codon 28 and 29, or in the preCore promoter region (see Figure 1).

Said probes being characterized in that they can function in a method as set out above.

An additional embodiment of the pr sent inv ntion includes at least one probe, pref rably at least two probes, characterizing the presence of a vaccine escape mutation in codon positions chosen from the HBsAg region of HBV, more particularly from the list of codons susceptible to mutations in the HBV HBsAg

region, such as at codons 122, 126, 141, 143, 144 or 145 (see Figure 1).

An additional embodiment of the present invention includes at least one probe, preferably at least two probes, characterizing the presence of a mutation in the RT pol gene region of HBV, that gives rise to resistance to drugs such as lamivudine and penciclovir, for instance mutation of M to V or to I at position 552 (in the YMDD motif), mutation of V to I at position 555, mutation of F to L at position 514, mutation of V to L at position 521, mutation of P to L at position 525 and mutation of L to M at position 528 (see Figure 1).

In a selected embodiment, a combination of at least two oligonucleotide probes is used and said combination of probes hybridizes specifically to at least two of the following groups of target sequences:

a mutant target sequence chosen from the HBV RT pol gene region,

a mutant target sequence chosen from the HBV preCore region,

a mutant target sequence chosen from the HBsAg region of HBV,

a HBV genotype-specific target sequence.

For instance, an embodiment involves hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from Figure 1.

Another selected embodiment involves, for instance, hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the RT pol gene region as represented in Figure 1.

Another selected embodiment involves, for instance, hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the pr Core region as represented in Figure 1.

Another selected embodiment involves, for instance, hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence

chosen from Figure 1 and at l ast one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence within the HBsAg region as represented in Figure 1.

In a selected embodiment, a combination of at least three oligonucleotide probes is used and said combination of probes hybridizes specifically to at least three of the following groups of target sequences:

a mutant target sequence chosen from the HBV RT pol gene region,

a mutant target sequence chosen from the HBV preCore region,

a mutant target sequence chosen from the HBsAg region of HBV,

a HBV genotype-specific target sequence.

For instance, an embodiment involves hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the preCore region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence chosen from the HBsAg region as represented in Figure 1.

For instance, another embodiment involves hybridizing with at least one probe hybridizing specifically to a mutant target sequence from the HBV RT pol gene region of HBV, and at least one probe hybridizing specifically to a mutant target sequence from the HBsAg region of HBV, and at least one probe hybridizing specifically to a genotype-specific target sequence from the HBsAg region of HBV. According to this embodiment, the relevant part of the HBV genome can be amplified by use of one primer pair, for instance HBPr 75 and HBPr 94.

In a selected embodiment, a combination of at least four oligonucleotide probes is used and said combination of probes hybridizes specifically to all of the following groups of target sequences:

a mutant target s quence chosen from the HBV RT pol gene region,

a mutant target sequence chosen from the HBV preCore region,

a mutant target sequence chosen from the HBsAg region of HBV,

a HBV genotype-specific target sequence.

Particularly preferred embodiments of the invention thus include a set of probes as set out above, comprising at least one, preferably at least two, at least three, at least four or more probe(s) for targeting one, preferably two, three or more nucleotide changes appearing in the alignment of HBV genomes as represented in Figure 1.

Even more preferred selected sets of probes according to the present invention include probes derived from two of the same or different regions of HBV bearing HBV mutated nucleotides, or in addition also a third (set of) probe(s) characterizing the presence of a third HBV mutation at any of the positions shown in Figure 1, or particular combinations thereof.

Particularly preferred is also a set of probes which allows simultaneous detection of HBV mutations at codons 15, 28 and 29 in the preCore region, possibly in combination with mutations in the preCore promoter regions, in combination with mutations at codons 122, 126, 141, 143, 144, 145 in the HBsAg region, possibly also in combination with mutations in the HBV pol gene at codons 514, 521, 525, 528, 552 or 555.

In the instances where the alignment of HBV genomes of Figure 1 is referred to in this invention, it should be construed as referring to an alignment of all existing and future HBV genomes. The existing HBV genome sequences can be deduced from any database, such as the EMBL/NCBI/DDBJ/GENBANK database.

A preferred set of preCore, preS1, HBsAg and RT pol gene probes of this invention are the probes with SEQ ID NO 1 to 278 of Table 1 (see also Figure 1).

Particularly preferred sets of probes in this respect are shown in Figure 2 and in Figure 4. The probes in Figure 2 and in Figure 4 were withheld after a first selection for preCore, preS1, HBsAg and RT pol probes.

The probes of the invention are designed for obtaining optimal performance under the same hybridization conditions so that they can be used in sets of at least 2 probes for simultaneous hybridization. This highly increases the usefulness of these probes and results in a significant gain in time and labour. Evidently, when other hybridization conditions would be preferred, all probes should be adapted accordingly by adding or deleting a number of nucleotides at their extremities. It

should be understood that these concomitant adaptations should give rise to essentially the same result, namely that the respective probes still hybridize specifically with the defined target. Such adaptations might also be necessary if the amplified material should be RNA in nature and not DNA as in the case for the NASBA system.

The selection of the preferred probes of the present invention is based on a reverse hybridization assay format using immobilized oligonucleotide probes present at distinct locations on a solid support. More particularly the selection of preferred probes of the present invention is based on the use of the Line Probe Assay (LiPA) principle which is a reverse hybridization assay using oligonucleotide probes immobilized as parallel lines on a solid support strip (Stuyver et al. 1993; international application WO 94/12670). This approach is particularly advantageous since it is fast and simple to perform. The reverse hybridization format and more particularly the LiPA approach has many practical advantages as compared to other DNA techniques or hybridization formats, especially when the use of a combination of probes is preferable or unavoidable to obtain the relevant information sought.

It is to be understood, however, that any other type of hybridization assay or format using any of the selected probes as described further in the invention, is also covered by the present invention.

The reverse hybridization approach implies that the probes are immobilized to certain locations on a solid support and that the target DNA is labelled in order to enable the detection of the hybrids formed.

The following definitions serve to illustrate the terms and expressions used in the present invention.

The term "genetic analysis" refers to the study of the nucleotide_sequence of the genome of HBV by any appropriate technique.

The term "HBV mutant" refers to any HBV strain harbouring genomic variations with serological, genetical or clinical consequences.

The term "vaccine escape mutant" is reviewed in the introduction section and in Example 7. The most important region lies between amino acid 124 and 147 of the HBsAg region, but can be extended from amino acid 114 to 150.

The term "mutant resistant to drugs such as lamivudine and penciclovir" is reviewed in the introduction section and in Example 8.

The term "HBV genotype" refers to HBV strains with an intergenotype variation of 8% or more based on a comparison of complete genomes.

The target material in the samples to be analyzed may either be DNA or RNA, e.g. genomic DNA, messenger RNA, viral RNA or amplified versions thereof. These molecules are also termed polynucleic acids.

It is possible to use genomic DNA or RNA molecules from samples susceptible of containing HBV in the methods according to the present invention. Well-known extraction and purification procedures are available for the isolation of RNA or DNA from a sample (f.i. in Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press (1989)).

The term "probe" refers to single stranded sequence-specific oligonucleotides which have a sequence which is complementary to the target sequence to be detected.

The term "target sequence" as referred to in the present invention describes the nucleotide sequence of a part of wild-type, polymorphic or mutant HBV gene sequence to be specifically detected by a probe according to the present invention. The polymorphic sequence may encompass one or more polymorphic nucleotides; the mutant sequence may encompass one or more nucleotides that are different from the wild-type sequence. It is to be understood that the term "mutant target sequence" not only covers the sequence containing a mutation, but also the corresponding wild-type sequence. Target sequences may generally refer to single nucleotide positions, codon positions, nucleotides encoding amino acids or to sequences spanning any of the foregoing positions. In the present invention said target sequence often includes one, two or more variable nucleotide positions. In the present invention polynucleic acids detected by the probes of the invention will comprise the target sequence against which the probe is det cted.

It is to be understood that the complement of said target sequence is also a suitable target sequence in some cases. The target sequences as defined in the present invention provide sequences which should at least be complementary to

the central part of the probe which is designed to hybridize specifically to said target region. In most cases the target sequence is completely complementary to the sequence of the probe.

The term "complementary" as used herein means that the sequence of the single stranded probe is exactly the (inverse) complement of the sequence of the single-stranded target, with the target being further defined as the sequence where the mutation to be detected is located.

Since the current application requires the detection of single basepair mismatches, stringent conditions for hybridization are required, allowing in principle only hybridization of exactly complementary sequences. However, variations are possible in the length of the probes (see below). It should also be noted that, since the central part of the probe is essential for its hybridization characteristics, possible deviations of the probe sequence versus the target sequence may be allowable towards head and tail of the probe when longer probe sequences are used. These variations, which may be conceived from the common knowledge in the art, should however always be evaluated experimentally, in order to check if they result in equivalent hybridization characteristics as the exactly complementary probes.

Preferably, the probes of the invention are about 5 to 50 nucleotides long, more preferably from about 10 to 25 nucleotides. Particularly preferred lengths of probes include 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides. The nucleotides as used in the present invention may be ribonucleotides, deoxyribonucleotides and modified nucleotides such as inosine or nucleotides containing modified groups which do not essentially alter their hybridisation characteristics.

Probe sequences are represented throughout the specification as single stranded DNA oligonucleotides from the 5' to the 3' end. It is obvious to the man skilled in the art that any of the below-specified probes can be used as such, or in their complementary form, or in their RNA form (wherein T is replaced by U).

The probes according to the invention can be prepared by cloning of recombinant plasmids containing inserts including the corr sponding nucleotide

sequences, if need be by cleaving the latter out from the cloned plasmids upon using the adequate nucleases and recovering them, e.g. by fractionation according to molecular weight. The probes according to the present invention can also be synthesized chemically, for instance by the conventional phospho-triester method.

The term "solid support" can refer to any substrate to which an oligonucleotide probe can be coupled, provided that it retains its hybridization characteristics and provided that the background level of hybridization remains low. Usually the solid substrate will be a microtiter plate, a membrane (e.g. nylon or nitrocellulose) or a microsphere (bead) or a chip. Prior to application to the membrane or fixation it may be convenient to modify the nucleic acid probe in order to facilitate fixation or improve the hybridization efficiency. Such modifications may encompass homopolymer tailing, coupling with different reactive groups such as aliphatic groups, NH_2 groups, SH groups, carboxylic groups, or coupling with biotin, haptens or proteins.

The term "labelled" refers to the use of labelled nucleic acids. Labelling may be carried out by the use of labelled nucleotides incorporated during the polymerase step of the amplification such as illustrated by Saiki et al. (1988) or Bej et al. (1990) or labelled primers, or by any other method known to the person skilled in the art. The nature of the label may be isotopic (^{32}P, ^{35}S, etc.) or non-isotopic (biotin, digoxigenin, etc.).

The term "primer" refers to a single stranded oligonucleotide sequence capable of acting as a point of initiation for synthesis of a primer extension product which is complementary to the nucleic acid strand to be copied. The length and the sequence of the primer must be such that they allow to prime the synthesis of the extension products. Preferably the primer is about 5-50 nucleotides long. Specific length and sequence will depend on the complexity of the required DNA or RNA targets, as well as on the conditions of primer use such as temperature and ionic strength.

The expression "suitable primer pair" in this invention refers to a pair of primers allowing the amplification of part or all of the HBV gene for which probes are immobilized.

The fact that amplification primers do not have to match exactly with the corresponding template sequence to warrant proper amplification is amply documented in the literature (Kwok et al., 1990).

The amplification method used can be either polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landgren et al., 1988; Wu & Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA; Guatelli et al., 1990; Compton, 1991), transcription-based amplification system (TAS; Kwoh et al., 1989), strand displacement amplification (SDA; Duck, 1990; Walker et al., 1992) or amplification by means of Qß replicase (Lizardi et al., 1988; Lomeli et al., 1989) or any other suitable method to amplify nucleic acid molecules known in the art.

The oligonucleotides used as primers or probes may also comprise nucleotide analogues such as phosphorothiates (Matsukura et al., 1987), alkylphosphorothiates (Miller et al., 1979) or peptide nucleic acids (Nielsen et al., 1991; Nielsen et al., 1993) or may contain intercalating agents (Asseline et al., 1984).

As most other variations or modifications introduced into the original DNA sequences of the invention these variations will necessitate adaptions with respect to the conditions under which the oligonucleotide should be used to obtain the required specificity and sensitivity. However the eventual results of hybridisation will be essentially the same as those obtained with the unmodified oligonucleotides.

The introduction of these modifications may be advantageous in order to positively influence characteristics such as hybridization kinetics, reversibility of the hybrid-formation, biological stability of the oligonucleotide molecules, etc.

The "sample" may be any biological material taken either directly from the infected human being (or animal), or after culturing (enrichment). Biological material may be e.g. expectorations of any kind, broncheolavages, blood, skin tissue, biopsies, sperm, lymphocyte blood culture material, colonies, liquid cultures, faecal samples, urine etc.

The sets of probes of the present invention will include at least 2, 3, 4, 5,

6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more probes. Said probes may be applied in two or more (possibly as many as there are probes) distinct and known positions on a solid substrate. Often it is preferable to apply two or more probes together in one and the same position of said solid support.

For designing probes with desired characteristics, the following useful guidelines known to the person skilled in the art can be applied.

Because the extent and specificity of hybridization reactions such as those described herein are affected by a number of factors, manipulation of one or more of those factors will determine the exact sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The importance and effect of various assay conditions, explained further herein, are known to those skilled in the art.

The stability of the [probe : target] nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long AT-rich sequences, by terminating the hybrids with G:C base pairs, and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %GC result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The base composition of the probe is significant because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs due to additional hydrogen bonding. Thus, hybridization involving complementary nucleic acids of higher G-C content will be stable at higher temperatures.

Conditions such as ionic strength and incubation temperature under which a probe will be used should also be taken into account when designing a probe. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of the hybrids will increase with increasing ionic strength. On the other hand, chemical reagents, such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, will increase the stringency of hybridization. Destabilization of the hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic

oligonucleotide probes of about 10-50 bases in length occurs approximately 5°C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

It is desirable to have probes which hybridize only under conditions of high stringency. Under high stringency conditions only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands forming a hybrid. The degree of stringency is chosen such as to maximize the difference in stability between the hybrid formed with the target and the nontarget nucleic acid. In the present case, single base pair changes need to be detected, which requires conditions of very high stringency.

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly complementary base sequence will normally primarily determine hybrid stability. While oligonucleotide probes of different lengths and base composition may be used, preferred oligonucleotide probes of this invention are between about 5 to 50 (more particularly 10-25) bases in length and have a sufficient stretch in the sequence which is perfectly complementary to the target nucleic acid sequence.

Regions in the target DNA or RNA which are known to form strong internal structures inhibitory to hybridization are less preferred. Likewise, probes with extensive self-complementarity should be avoided. As explained above, hybridization is the association of two single strands of complementary nucleic acids to form a hydrogen bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in a hybrid that it will be less able to

participate in formation of a new hybrid. There can be intramolecular and intermolecular hybrids formed within the molecules of one type of probe if there is sufficient self complementarity. Such structures can be avoided through careful probe design. By designing a probe so that a substantial portion of the sequence of interest is single stranded, the rate and extent of hybridization may be greatly increased. Computer programs are available to search for this type of interaction. However, in certain instances, it may not be possible to avoid this type of interaction.

Standard hybridization and wash conditions are disclosed in the Materials & Methods section of the Examples. Other conditions are for instance 3X SSC (Sodium Saline Citrate), 20% deionized FA (Formamide) at 50°C.

Other solutions (SSPE (Sodium saline phosphate EDTA), TMACl (Tetramethyl ammonium Chloride), etc.) and temperatures can also be used provided that the specificity and sensitivity of the probes is maintained. If need be, slight modifications of the probes in length or in sequence have to be carried out to maintain the specificity and sensitivity required under the given circumstances.

In a more preferential embodiment, the above-mentioned polynucleic acids from step (i) or (ii) are hybridized with at least two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more of the above-mentioned target region specific probes, preferably with 5 or 6 probes, which, taken together, cover the "mutation region" of the relevant HBV gene.

The term "mutation region" means the region in the relevant HBV gene sequence where at least one mutation encoding a HBV mutant is located in a preferred part of this mutation region is represented in figure 1.

Apart from mutation regions as defined above the HBV wild-type or mutant genomes may also show polymorphic nucleotide variations at positions other than those referred to as genotype specific or mutant specific variated positions as shown in Figure 1.

Since some mutations may be more frequently occurring than others, e.g. in certain geographic areas or in specific circumstanc s (e.g. rather closed

communities) it may be appropriate to screen only for specific mutations, using a selected set of probes as indicated above. This would result in a more simple test, which would cover the needs under certain circumstances.

In order to detect HBV genotypes and/or HBV mutants with the selected set of oligonucleotide probes, any hybridization method known in the art can be used (conventional dot-blot, Southern blot, sandwich, etc.).

However, in order to obtain fast and easy results if a multitude of probes are involved, a reverse hybridization format may be most convenient.

In a preferred embodiment the selected set of probes are immobilized to a solid support in known distinct locations (dots, lines or other figures). In another preferred embodiment the selected set of probes are immobilized to a membrane strip in a line fashion. Said probes may be immobilized individually or as mixtures to delineated locations on the solid support.

A specific and very user-friendly embodiment of the above-mentioned preferential method is the LiPA method, where the above-mentioned set of probes is immobilized in parallel lines on a membrane, as further described in the examples.

The invention also provides for a set of primers allowing amplification of the region of the respective HBV gene to be detected by means of probes. Examples of such primers of the invention are given in Table 1 and Figure 1.

Primers may be labelled with a label of choice (e.g. biotine). Different primer-based target amplification systems may be used, and preferably PCR-amplification, as set out in the examples. Single-round or nested PCR may be used.

The invention also provides a kit for detection and/or genetic analysis of HBV genotypes and/or HBV mutants present in a biological sample comprising the following components:

(i) when appropriate, a means for releasing, isolating or concentrating the polynucleic acids present in said sample;

(ii) when appropriate, at least one suitable primer pair;

(iii) at least two of the probes as defined above, possibly fixed to a solid support;

(iv) a hybridization buffer, or components necessary for producing said buffer;

(v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

(vii) when appropriate, a means for attaching said probe to a known location on solid support.

The term "hybridization buffer" means a buffer enabling a hybridization reaction to occur between the probes and the polynucleic acids present in the sample, or the amplified products, under the appropriate stringency conditions.

The term "wash solution" means a solution enabling washing of the hybrids formed under the appropriate stringency conditions.

As illustrated in the Examples section, a line probe assay (LiPA) was designed for screening for HBV genotypes and/or HBV mutants. The principle of the assay is based on reverse hybridization of an amplified polynucleic acid fragment such as a biotinylated PCR fragment of the HBV gene onto short oligonucleotides. The latter hybrid can then, via a biotine-streptavidine coupling, be detected with a non-radioactive colour developing system.

The following examples only serve to illustrate the present invention. These examples are in no way intended to limit the scope of the present invention.

FIGURE AND TABLE LEGENDS

Figure 1: Alignment of 35 complete HBV genomes. Isolates belonging to genotype A are: HBVXCPS, HBVADW, HVHEPB, S50225, HPBADWZCG; genotype B: HPBADW3, HPBADWZ, HPBADW1, HPBADW2; genotype C: HPBCGADR, HBVADRM, HPBADRA, HPBCG, HEHBVAYR, HBVADR, HBVADR4, HPBADR1C, HPBADRC, HBVPREX, HPBETNC, HHVBC, HHVCCHA; genotype D: HBVAYWMCG, HBVAYWC, HBVAYWCI, HBVAYWE, HBVDNA, HPBHBVAA, XXHEPAV, HBVORFS; genotype E: HHVBE4, HHVBBAS; and genotype F: HHBF, HHVBFFOU, HBVADW4A. To preserve alignment, several gaps were created in the alignment and are indicated with /. Positions of start and end of the different HBV encoded genes is indicated: HBsAg: hepatitis B surface antigen (small surface antigen); HBx: hepatitis B X protein; HB Pol: hepatits B polymerase protein, encoding a terminal protein, a spacer, a RT/DNA polymerase region, and an RNAse H activity; HBcAg: hepatitis B Core antigen; HBpreS1Ag: hepatitis B preS1 antigen (large surface antigen); HBpreS2Ag: hepatitis B preS2 antigen (middle surface antigen). The position of the PCR primers is indicated with a large box over all 35 sequences. The polarity of the PCR primer can be deduced from the position of the name above these boxes: left = antisense primer; right = sense primer. LiPA probes are indicated with small boxes, the numbers of the probes are indicated next to the probes or to the right of the alignment, and correspond to the probe numbers in Table 1.

Figure 2: LiPA HBV design. The content of a HBV LiPA strip is detailed. For each line number, the region on the viral genome is indicated, together with the genotype that is detected, the probe number that corresponds with the boxes from the alignment in Figure 1, and the sequence of the probe.

Figure 3: Combined result of genotype determination in the preS1 region and preCore scanning on 24 samples. The interpretation of each sample is given under each strip. Probe reactivities on lines 3 to 14 are obtained from the preS1 PCR

fragment, probe reactivities on lines 15 to 27 are due to the preCore PCR fragment. Genotypes are indicated from A to F. The interpretation for the preCore region is as follows: W = wild type; M = mutant; I = indeterminate, meaning that no reactivity is observed, which is due to mutations that could not yet be detected with the selected probes; mix = mixture of wild type and mutant; interpretation of codon 15 is only relevant for genotype A, the absence of reactivity on HBPr 45 for genotypes B to F is of no use as is indicated with - (not applicable). Since the presence or absence of preCore mutations has effect on the serological HBeAg status, this is also indicated.

Figure 4: Probes used in HBV LiPA. Probes were designed for genotyping in the HBsAg region and for detection of drug resistance mutations in the YMDD motif (see also Figure 5), as well as for detection of mutations in the pre Core region (see also Figure 6).

Figure 5: Example of a LiPA assay combining HBV genotyping in the HBsAg region and detection of drug resistance mutations in the YMDD motif. Genotypes are indicated from A to F. The design of the strip is shown to the right, with the numbers of the probes corresponding to the numbers in Table 1 and in Figure 4. The genotypes and mutant motifs to which each probe hybridizes are written to the outer right. The combination of reactive probes allows the determination of a unique genotype.

Figure 6: Example of the determination of preCore mutations by the LiPA technique. The design of the strip is shown to the right, with the numbers of the probes corresponding to the numbers in Table 1. The mutant target sequences to which the probes hybridize are indicated to the outer right. Motif M2 corresponds to a mutation in codon 28, M4 corresponds to a mutation in codon 29. M2/M4 has mutations in both 28 and 29.

Figure 7: Detection of a mutation in the YMDD motif of HBV pol upon treatment

with lamivudune. The graph shows a time course of the viral load during lamivudine treatment. To the right LiPA strips are shown, corresponding to assays at the beginning of the treatment (5/95), 10 months of treatment (2/96) and 14 months of treatment (6/96). The assay shows that during treatment the YMDD motif mutates to YVDD.

Table 1: Overview of all primers and probes referred to in the Figures with an indication of their respective SEQ ID NO and the region of the HBV genome they are designed for. Primers from the PreS1 region include 1, 106, 2 (sense primers) and 4, 107 and 3 (antisense primers). Primers from the HBsAg region include 75 and 104 (sense primers) and 76, 94 and 105 (antisense primers). Primers from the PreCore region include 5, 6, 69, 70, 84, 86, 87 and108 (sense primers) and 7, 8, 85 and 109 (antisense primers). The remaining oligonucleotides are probes from the PreCore, PreS1, HBsAg and RT pol gene regions of HBV as indicated. The YMDDV motif and its mutants consist of amino acids 551 to 555 of the RT pol protein; the sequence MGVGL and its mutant consist of amino acids 519 to 523 of the RT pol protein; the sequence SPFLL and its mutants and genotypic variants consist of amino acids 524 to 528 of the RT pol protein.

Table 1 : HBV probe and primer design

Name	Sequence	SEQ ID NO	Region
HBPr1	GGGTCACCATATTCTTGGG	1	preS1 primer sense
HBPr2	GAACAAGAGCTACAGCATGGG	2	preS1 primer sense
HBPr3	CCACTGCATGGCCTGAGGATG	3	preS1 primer anti-sense
HBPr4	GTTCCT/GGAACTGGAGCCACCAG	4	preS1 primer anti-sense
HBPr5	TCTTTGTATTAGGAGGCTGTAG	5	preCore primer sense
HBPr6	GCTGTAGGCATAAATTGGTCTG	6	preCore primer sense
HBPr7	CTCCACAGT/AAGCTCCAAATTC	7	preCore primer anti-sense
HBPr8	GAAGGAAAGAAGTCAGAAGGC	8	preCore primer anti-sense
HBPr9	TGGCTTTGGGGCATGG	9	preCore
HBPr10	TGGCTTTAGGGCATGG	10	preCore
HBPr11	TGGCTTTAGGACATGG	11	preCore
HBPr12	AAGTTGCATGGTGCTG	12	preCore
HBPr13	CACCTCTGCCTAATCAT	13	preCore
HBPr14	TGGGGTGGAGCCCTCAG	14	preS1
HBPr15	GCCAGCAGCCAACCAG	15	preS1
HBPr16	CCCATGGGGGACTGT	16	preS1
HBPr17	AACCCCAACAAGGATG	17	preS1
HBPr18	TCCACCAGCAATCCT	18	preS1
HBPr19	TGGGGGAAGAATATTT	19	preS1
HBPr20	AAATTCCAGCAGTCCC	20	preS1
HBPr21	GTTCCCAACCCTCTGG	21	preS1
HBPr22	AACCTCGCAAAGGCAT	22	preS1
HBPr23	TGCATTCAAAGCCAAC	23	preS1
HBPr24	TACTCACAACTGTGCC	24	preS1
HBPr25	ACCCTGCGTTCGGAGC	25	preS1
HBPr26	CAGGAAGACAGCCTAC	26	preS1
HBPr27	GATCCAGCCTTCAGAG	27	preS1
HBPr28	ATGCTCCAGCTCCTAC	28	preS1
HBPr29	GCTTTCTTGGACGGTC	29	preS1
HBPr30	CTACCCCAATCACTCC	30	preS1
HBPr31	AGCACCTCTCTCAACG	31	preS1
HBPr32	CCAATGGCAAACAAGG	32	preS1
HBPr33	CTGAGGGCTCCACCCCA	33	preS1
HBPr34	ATGCAACTTTTTCACC	34	preCore
HBPr35	ATCTCTTGTACATGTC	35	preCore

HBPr36	ATCTCATGTTCATGTC	36	preCore
HBPr37	CAGTGGGACATGTACA	37	preCore
HBPr38	CAGTAGGACATGAACA	38	preCore
HBPr39	CTGTTCAAGCCTCCAA	39	preCore
HBPr40	AGCCTCCAAGCTGTGC	40	preCore
HBPr41	AAAGCCACCCAAGGCA	41	preCore
HBPr42	TGGCTTTAGGACATGGA	42	preCore
HBPr43	GACATGTACAAGAGATGA	43	preCore
HBPr44	GACATGAACATGAGATGA	44	preCore
HBPr45	TGTACATGTCCCACTGTT	45	preCore
HBPr46	TGTTCATGTCCTACTGTT	46	preCore
HBPr47	ACTGTTCAAGCCTCCAAG	47	preCore
HBPr48	GGCACAGGCTTGGAGGCTT	48	preCore
HBPr49	AAAGCCACCCAAGGCACA	49	preCore
HBPr50	CCCAGAGGGTTGGGAAC	50	preS1
HBPr51	CAGCATGGGGCAGAATCT	51	preS1
HBPr52	TCCACCAGCAATCCTCTG	52	preS1
HBPr53	GGATCCAGCCTTCAGAGC	53	preS1
HBPr54	TCAGGAAGACAGCCTAC	54	preS1
HBPr55	TTCAACCCCAACAAGGATC	55	preS1
HBPr56	AATGCTCCAGCTCCTAC	56	preS1
HBPr57	CTGCATTCAAAGCCAACT	57	preS1
HBPr58	CCCCATGGGGGACTGTTG	58	preS1
HBPr59	CATACTCACAACTGTGCCA	59	preS1
HBPr60	GGGCTTTCTTGGACGGTCC	60	preS1
HBPr61	CTCTCGAATGGGGGAAGA	61	preS1
HBPr62	CCTACCCCAATCACTCCA	62	preS1
HBPr63	AGCACCTCTCTCAACGACA	63	preS1
HBPr64	GCAAATTCCAGCAGTCCCG	64	preS1
HBPr65	GCCAATGGCAAACAAGGTA	65	preS1
HBPr66	GACATGAACATGAGATG	66	preCore
HBPr67	GGACATGAACAAGAGAT	67	preCore
HBPr68	GACATGTACAAGAGATG	68	preCore
HBPr69	ACATAAGAGGACTCTTGGAC	69	preCore primer sense
HBPr70	TACTTCAAAGACTGTGTGTTTA	70	preCore primer sense
HBPr71	ACAAAGACCTTTAAC/TCT	71	preCore promoter
HBPr72	ACAAAGATCATTAAC/TCT	72	preCore promoter
HBPr73	TTCCACCAGCAATCCTC	73	preS1
HBPr74	GATCCAGCCTTCAGAGC	74	preS1

HBPr75	CAAGGTATGTTGCCCGTTTGTCC	75	HBsAg primer sense
HBPr76	CCAAACAGTGGGGGAAAGCCC	76	HBsAg primer anti-sense
HBPr77	CTACGGATGGAAATTGC	77	HBsAg codon 145 wild type
HBPr78	TACGGACGGAAACTGC	78	HBsAg codon 145 wild type
HBPr79	TTCGGACGGAAACTGC	79	HBsAg codon 145 wild type
HBPr80	CTTCGGACGGAAATTGC	80	HBsAg codon 145 wild type
HBPr81	CTACGGATAGAAATTGC	81	HBsAg codon 145 mutant
HBPr82	CTTCGGACAGAAATTGC	82	HBsAg codon 145 mutant
HBPr83	CTATGGGAGTGGGCCTCAGT/CC	83	HB Pol
HBPr84	GCTGTAGGCATAAATTGGTCTG	84	preCore primer sense
HBPr85	CTCCACAGT/AAGCTCCAAATTC	85	preCore primer anti-sense
HBPr86	ACATAAGAGGACTCTTGGAC	86	preCore primer sense
HBPr87	TACTTCAAAGACTGTGTGTTTA	87	preCore primer sense
HBPr88	TAGGTTAAAGGTCTTTGT	88	preCore promoter
HBPr89	TAGGTTAATGATCTTTGT	89	preCore promoter
HBPr90	CATGTCCCACTGTTCAA	90	preCore
HBPr91	CATGTCCTACTGTTCAA	91	preCore
HBPr92	TTCTGCCCCATGCTGTA	92	preS1
HBPr93	TTCTGCCCCATGCTGTAG	93	preS1
HBPr94	GGTAA/TAAAGGGACTCAC/AGATG	94	HBsAg primer anti-sense
HBPr95	TCAGCTATATGGATGAT	95	HB Pol
HBPr96	CAGCTATATGGATGAT	96	HB Pol
HBPr97	TTCAGCTATATGGATG	97	HB Pol
HBPr98	TCAGTTATATGGATGAT	98	HB Pol
HBPr99	TTTCAGTTATATGGATG	99	HB Pol
HBPr100	TTTAGTTATATGGATGA	100	HB Pol
HBPr101	TCAGCTATGTGGATGAT	101	HB Pol
HBPr102	TCAGTTATGTGGATGAT	102	HB Pol
HBPr103	TTTCAGCTATGTGGATG	103	HB Pol
HBPr104	CAAGGTATGTTGCCCGTTTGTCC	104	HBsAg primer sense
HBPr105	GGT/CAA/TAAAGGGACTCAC/AGATG	105	HBsAg primer anti-sense
HBPr106	GGGTCACCATATTCTTGGG	106	preS1 primer sense
HBPr107	GTTCCT/GGAACTGGAGCCACCAG	107	preS1 primer anti-sense
HBPr108	CCGGAAAGCTTGAGCTCTTCTTTTTCACCTCTGCCTAATC	108	preCore primer sense
HBPr109	CCGGAAAGCTTGAGCTCTTCAAAAAGTTGCATGGTGCTGG	109	preCore primer anti-sense
HBPr110	CCTCTGCCGATCCATACTGCGGAAC	110	preX primer sense
HBPr111	CTGCGAGGCGAGGGAGTTCTTCTTC	111	HB Core primer anti-sense
HBPr112	TGCCATTTGTTCAGTGGTTCGTAGGGC	112	HBsAg primer sense
HBPr113	CCGGCAGATGAGAAGGCACAGACGG	113	HBX primer antisense

probes

HBPr114	TTCAGCTATATGGATGAT	114	YMDD motif
HBPr115	TCAGCTATATGGATGATG	115	YMDD motif
HBPr116	TTCAGCTATGTGGATGAT	116	YMDD motif
HBPr117	TCAGCTATGTGGATGATG	117	YMDD motif
HBPr118	GGCTTTGGGGCATGG	118	preCore codon 28 wild type
HBPr119	TGGCTTTGGGGCATG	119	preCore codon 28 wild type
HBPr120	GTGGCTTTGGGGCATG	120	preCore codon 28 wild type
HBPr121	GGCTTTGGGGCATGGA	121	preCore codon 28 wild type
HBPr122	TGGCTTTGGGACATGG	122	preCore codon 28 wild type, codon 29 mutant
HBPr123	GGCTTTGGGACATGG	123	preCore codon 28 wild type, codon 29 mutant
HBPr124	TGGCTTTGGGACATG	124	preCore codon 28 wild type, codon 29 mutant
HBPr125	GTGGCTTTGGGACATG	125	preCore codon 28 wild type, codon 29 mutant
HBPr126	GGCTTTGGGACATGGA	126	preCore codon 28 wild type, codon 29 mutant
HBPr127	TCAGTTATATGGATGATG	127	YMDD genotype D, wild tpye
HBPr128	TTCAGTTATATGGATGAT	128	YMDD genotype D, wild tpye
HBPr129	TTTCAGTTATATGGATGAT	129	YMDD genotype D, wild tpye
HBPr130	TCAGTTATGTGGATGATG	130	YMDD genotype D, mutant
HBPr131	TTCAGTTATGTGGATGAT	131	YMDD genotype D, mutant
HBPr132	TTTCAGTTATGTGGATGAT	132	YMDD genotype D, mutant
HBPr133	TTTCAGTTATGTGGATGA	133	YMDD genotype D, mutant
HBPr134	TGCTGCTATGCCTCATCTTC	134	outer HBsAg primer sense
HBPr135	CA(G/A)AGACAAAAGAAAATTGG	135	outer HBsAg primer anti-sense
HBPr136	CTATGGATGGAAATTGC	136	HBsAg mutant codon 143
HBPR137	CCTATGGATGGAAATTG	137	HBsAg mutant codon 143
HBPR138	ACCTATGGATGGAAATT	138	HBsAg mutant codon 143
HBPr139	CT CAA GGC AAC TCT ATG TGG	139	HBsAg, genotype A
HBPr140	CT CAA GGC AAC TCT ATG GG	140	HBsAg, genotype A
HBPr141	T CAA GGC AAC TCT ATG TTG	141	HBsAg, genotype A
HBPr142	ATC CCA TCA TCT TGG G	142	HBsAg, genotype B
HBPr143	ATC CCA TCA TCT TGG GCG G	143	HBsAg, genotype B
HBPr144	TC CCA TCA TCT TGG GCG G	144	HBsAg, genotype B
HBPr145	C CCA TCA TCT TGG GCT GG	145	HBsAg, genotype B
HBPr146	TTC GCA AAA TAC CTA TGG	146	HBsAg, genotype B
HBPr147	T TTC GCA AAA TAC CTA TG	147	HBsAg, genotype B
HBPr148	CT TTC GCA AAA TAC CTA TG	148	HBsAg, genotype B
HBPr149	TC GCA AAA TAC CTA TGG G	149	HBsAg, genotype B
HBPr150	T CTA CTT CCA GGA ACA T	150	HBsAg, genotype C
HBPr151	T CTA CTT CCA GGA ACA TC	151	HBsAg, genotype C
HBPr152	CT CTA CTT CCA GGA ACA T	152	HBsAg, genotype C

HBPr153	CT CTA CTT CCA GGA ACA G	153	HBsAg, genotype C
HBPr154	C TGC ACG ATT CCT GCT	154	HBsAg, genotype C
HBPr155	TGC ACG ATT CCT GCT CA	155	HBsAg, genotype C
HBPr156	C TGC ACG ATT CCT GCT C	156	HBsAg, genotype C
HBPr157	TGC ACG ATT CCT GCT CAA	157	HBsAg, genotype C
HBPr158	TTC GCA AGA TTC CTA TG	158	HBsAg, genotype C
HBPr159	CT TTC GCA AGA TTC CTA T	159	HBsAg, genotype C
HBPr160	CT TTC GCA AGA TTC CTA	160	HBsAg, genotype C
HBPr161	CT TTC GCA AGA TTC CTA TG	161	HBsAg, genotype C
HBPr162	C TCT ATG TAT CCC TCC T	162	HBsAg, genotype D
HBPr163	TCT ATG TAT CCC TCC TG	163	HBsAg, genotype D
HBPr164	C TCT ATG TAT CCC TCC TGG	164	HBsAg, genotype D
HBPr165	CC TCT ATG TAT CCC TCC T	165	HBsAg, genotype D
HBPr166	C TGT ACC AAA CCT TCG G	166	HBsAg, genotype D
HBPr167	C TGT ACC AAA CCT TCG	167	HBsAg, genotype D
HBPr168	GC TGT ACC AAA CCT TCG G	168	HBsAg, genotype D
HBPr169	TGT ACC AAA CCT TCG GAG	169	HBsAg, genotype D
HBPr170	GGA CCC TGC CGA ACC T	170	HBsAg, genotype E
HBPr171	GGA CCC TGC CGA ACC G	171	HBsAg, genotype E
HBPr172	G GGA CCC TGC CGA AC	172	HBsAg, genotype E
HBPr173	GGA CCC TGC CGA AC	173	HBsAg, genotype E
HBPr174	GT TGC TGT TCA AAA CCT T	174	HBsAg, genotype E
HBPr175	GT TGC TGT TCA AAA CCT G	175	HBsAg, genotype E
HBPr176	TGT TGC TGT TCA AAA CCT G	176	HBsAg, genotype E
HBPr177	A TGT TGC TGT TCA AAA CCT G	177	HBsAg, genotype E
HBPr178	GA TCC ACG ACC ACC A	178	HBsAg, genotype F
HBPr179	GGA TCC ACG ACC ACC A	179	HBsAg, genotype F
HBPr180	GGA TCC ACG ACC ACC	180	HBsAg, genotype F
HBPr181	GA TCC ACG ACC ACC AGG	181	HBsAg, genotype F
HBPr182	TGT TCC AAA CCC TCG G	182	HBsAg, genotype F
HBPr183	C TGT TCC AAA CCC TCG	183	HBsAg, genotype F
HBPr184	C TGT TCC AAA CCC TCG G	184	HBsAg, genotype F
HBPr185	GT TCC AAA CCC TCG GAT	185	HBsAg, genotype F
HBPr186	G CCA AAT CTG TGC AGC	186	HBsAg, genotype F
HBPr187	CCA AAT CTG TGC AGC AT	187	HBsAg, genotype F
HBPr188	G CCA AAT CTG TGC AGC AG	188	HBsAg, genotype F
HBPr189	GG CCA AAT CTG TGC AGC	189	HBsAg, genotype F
HBPr190	A TCA ACA ACA ACC AGT A	190	HBsAg, genotype A
HBPr191	GA TCA ACA ACA ACC AGT	191	HBsAg, genotype A

HBPr192	GA TCA ACA ACA ACC AGT A	192	HBsAg, genotype A
HBPr193	GGA TCA ACA ACA ACC AGT	193	HBsAg, genotype A
HBPr194	T CAA GGC AAC TCT ATG TGG	194	HBsAg, genotype A
HBPr195	AGG TTA AAG GTC TTT GT	195	promoter genotype A wild type
HBPr196	T AGG TTA AAG GTC TTT GG	196	promoter genotype A wild type
HBPr197	TT AGG TTA AAG GTC TTT	197	promoter genotype A wild type
HBPr198	GG TTA AAG GTC TTT GTA GG	198	promoter genotype A wild type
HBPr199	AGG TTA ATG ATC TTT GT	199	promoter genotype A mutant
HBPr200	T AGG TTA ATG ATC TTT GG	200	promoter genotype A mutant
HBPr201	CT TTC GCA AGA TTC CTA TGG	201	HBsAg genotype C codon 160
HBPr202	GCT TTC GCA AGA TTC CTA TG	202	HBsAg genotype C codon 160
HBPr203	GCT TTC GCA AGA TTC CTA TGG	203	HBsAg genotype C codon 160
HBPr204	CT TTC GCA AGA TTC CTA TGG G	204	HBsAg genotype C codon 160
HBPr205	GC TGT ACC AAA CCT TCG GAG	205	HBsAg genotype D codon 140
HBPr206	TGC TGT ACC AAA CCT TCG G	206	HBsAg genotype D codon 140
HBPr207	TGC TGT ACC AAA CCT TCG GAG	207	HBsAg genotype D codon 140
HBPr208	GC TGT ACC AAA CCT TCG GAT	208	HBsAg genotype D codon 140
HBPr209	TGG TTC GCC GGG CTT T	209	HBsAg genotype E codon 184
HBPr210	G TGG TTC GCC GGG CTT G	210	HBsAg genotype E codon 184
HBPr211	GG TTC GCC GGG CTT TC	211	HBsAg genotype E codon 184
HBPr212	TGG TTC GCC GGG CTT TC	212	HBsAg genotype E codon 184
HBPr213	AG TGG TTC GCC GGG CTG G	213	HBsAg genotype E codon 184
HBPr214	A GGA TCC ACG ACC ACC AGG	214	HBsAg genotype F
HBPr215	A GGA TCC ACG ACC ACC AGT	215	HBsAg genotype F
HBPr216	CA GGA TCC ACG ACC ACC AGG	216	HBsAg genotype F
HBPr217	C TGT TCC AAA CCC TCG GAG	217	HBsAg genotype F
HBPr218	C TGT TCC AAA CCC TCG GAT	218	HBsAg genotype F
HBPr219	GC TGT TCC AAA CCC TCG GAG	219	HBsAg genotype F
HBPr220	CTGAACCTTTACCCCGTTGC	220	enhancer primer
HBPr221	CTCGCCAACTTACAAGGCCTTTC	221	enhancer primer
HBPr222	AGAATGGCTTGCCTGAGTGC	222	Core primer anti-sense
HBPr223	GCT TTC GCA AGA TTC CTA TGG G	223	HBsAg genotype C codon 160
HBPr224	G GCT TTC GCA AGA TTC CTA TGG	224	HBsAg genotype C codon 160
HBPr225	G GCT TTC GCA AGA TTC CTA TGG G	225	HBsAg genotype C codon 160
HBPr226	G GCT TTC GCA AGA TTC CTA TGG GA	226	HBsAg genotype C codon 160
HBPr227	C AGC TAT ATG GAT GAT GTG	227	YMDDV motif
HBPr228	AGC TAT ATG GAT GAT GTG GG	228	YMDDV motif
HBPr229	GC TAT ATG GAT GAT GTG GT	229	YMDDV motif
HBPr230	AGC TAT ATG GAT GAT GTG GT	230	YMDDV motif

HBPr231	C AGC TAT ATG GAT GAT ATA	231	YMDDI MOTIF
HBPr232	AGC TAT ATG GAT GAT ATA GG	232	YMDDI MOTIF
HBPr233	GC TAT ATG GAT GAT ATA GT	233	YMDDI MOTIF
HBPr234	AGC TAT ATG GAT GAT ATA GT	234	YMDDI MOTIF
HBPr235	CCA TCA TCT TGG GCT TG	235	HBSAg GENOTYPE B CODON 155
HBPr236	CA TCA TCT TGG GCT TT	236	HBSAg GENOTYPE B CODON 155
HBPr237	CCA TCA TCT TGG GCT TT	237	HBSAg GENOTYPE B CODON 155
HBPr238	CCA TCA TCT TGG GCT TTC	238	HBSAg GENOTYPE B CODON 155
HBPr239	CCC ACT GTC TGG CTT TC	239	HBSAg GENOTYPE B CODON 190
HBPr240	CC ACT GTC TGG CTT TC	240	HBSAg GENOTYPE B CODON 190
HBPr241	CC ACT GTC TGG CTT T	241	HBSAg GENOTYPE B CODON 190
HBPr242	CCC ACT GTC TGG CTT G	242	HBSAg GENOTYPE B CODON 190
HBPr243	TAT ATG GAT GAT GTG GTA	243	YMDDV MOTIF
HBPr244	TAT GTG GAT GAT GTG GTA	244	YVDDV MOTIF
HBPr245	TAT ATA GAT GAT GTG GTA	245	YIDDV MOTIF
HBPr246	TAT ATT GAT GAT GTG GTA	246	YIDDV MOTIF
HBPr247	TAT GTA GAT GAT GTG GTA	247	YVDDV MOTIF
HBPr248	TAT GTT GAT GAT GTG GTA	248	YVDDV MOTIF
HBPr249	TAT ATG GAT GAT ATA GTA	249	YMDDI MOTIF
HBPr250	TAT ATG GAT GAT ATC GTA	250	YMDDI MOTIF
HBPr251	TAT GTG GAT GAT ATA GTA	251	YVDDI MOTIF
HBPr252	TAT GTG GAT GAT ATC GTA	252	YVDDI MOTIF
HBPr253	TAT ATA GAT GAT ATA GTA	253	YIDDI MOTIF
HBPr254	TAT ATA GAT GAT ATC GTA	254	YIDDI MOTIF
HBPr255	TAT ATT GAT GAT ATA GTA	255	YIDDI MOTIF
HBPr256	TAT ATT GAT GAT ATC GTA	256	YIDDI MOTIF
HBPr257	TAT GTA GAT GAT ATA GTA	257	YVDDI MOTIF
HBPr258	TAT GTA GAT GAT ATC GTA	258	YVDDI MOTIF
HBPr259	TAT GTT GAT GAT ATA GTA	259	YVDDI MOTIF
HBPr260	TAT GTT GAT GAT ATC GTA	260	YVDDI MOTIF
HBPr261	TAT ATG GAT GAT CTG GTA	261	YMDDL MOTIF
HBPr262	TAT GTG GAT GAT CTG GTA	262	YVDDL MOTIF
HBPr263	TAT ATA GAT GAT CTG GTA	263	YIDDL MOTIF
HBPr264	TAT ATT GAT GAT CTG GTA	264	YIDDL MOTIF
HBPr265	TAT GTA GAT GAT CTG GTA	265	YVDDL MOTIF
HBPr266	TAT GTT GAT GAT CTG GTA	266	YVDDL MOTIF
HBPr267	T ATG GGA GTG GGC CTC AG	267	MGVGL
HBPr268	T ATG GGA TTG GGC CTC AG	268	MGLGL
HBPr269	C AGT CCG TTT CTC TTG GC	269	SPFLL

EXAMPLES

Example 1. HBV DNA preparation and PCR amplification

Serum samples were collected from HBsAg-positive individuals and stored at minus 20°C until use in 0.5 ml aliquots. To prepare the viral genome, 18 μl serum was mixed with 2 μl 1N NaOH and incubated at 37°C for 60 minutes. The denaturation was stopped and neutralized by adding 20 μl of 0.1N HCl. After a 15 minutes centrifugation step, the supernatant was collected and the pellet discarded. PCR was carried out on this lysate as follows: 32 μl H_2O was mixed with 5 μl of 10x PCR buffer, 1 μl 10 mM dXTPs, 1 μl of each biotinylated primer (10 pmol/μl), 10 μl of serum lysate, and 2 U Taq enzyme. The amplification scheme contained 40 cycles of 95°C 1 min, annealing at 45°C for 1 min, and extension at 72°C for 1 min. Amplification products were visualized on 3% agarose gel.

The outer primer set for preS1 has the following sequence:
outer sense: HBPr 1: 5'-bio-GGGTCACCATATTCTTGGG- 3'
outer antisense HBPr 4: 5'-bio-GTTCC(T/G)GAACTGGAGCCACCAG-3'
The outer primer set for preCore has the following sequence:
outer sense: HBPr 69: 5'-bio-ACATAAGAGGACTCTTGGAC-3'
outer antisense: HBPr 8: 5'-bio-GAAGGAAAGAAGTCAGAAGGC-3'
The outer primer set for HBsAg has the following sequence:
outer sense: HBPr 134: 5'-bio-TGCTGCTATGCCTCATCTTC-3'
outer antisense: HBPr 135: 5'-bio-CA(G/A)AGACAAAAGAAAATTGG-3'.

Samples that were negative in the first round PCR were retested in a nested reaction composed of the following: μl H_2O, 5 μl 10x Taq buffer, 1 μl 10 mM dXTPs, 1 μl of each nested primer (10 pmol/μl), 1 μl of the first round PCR product, and 2 U Taq polymerase. The amplification scheme was identical as for the first round PCR. The sequence of the nested primers were as follows, for the preS1 region:

nested sense HBPr 2: 5'-bio-GAACAAGAGCTACAGCATGGG- 3'
nested antisense HBPr 3: 5'-bio-CCACTGCATGGCCTGAGGATG-3';

and for the preCore region:

nested sense HBPr 70: 5'-bio-TACTTCAAAGACTGTGTGTTTA-3'

nested antisense HBPr 7: 5'-bio- CTCCACAG(T/A)AGCTCCAAATTC-3'

In a second reaction the HBsAg region can be amplified in a similar protocol by using the following primers: HBPr 75: 5'-bio-CAAGGTATGTTGCCCGTTTGTCC-3' in combination with either HBPr 76: 5'-bio-CCAAACAGTGGGGGAAAGCCC-3'; or with HBPr 94: 5'-bio-GGTA(A/T)AAAGGGACTCA(C/A)GATG-3'.

Example 2. Preparation of the Line Probe Assays

Probes were designed to cover the universal, genotypic and mutant motifs. In principle only probes that discriminate between one single nucleotide variation were retained. However, for certain polymorphisms at the extreme ends of the probe, cross-reactivity was tolerated. Specificity was reached experimentally for each probe individually after considering the % (G+C), the probe length, the final concentration, and hybridization temperature. Optimized probes were provided enzymatically with a poly-T-tail using the TdT (Pharmacia) in a standard reaction condition. Briefly, 400 pmol probe was incubated at 37°C in a 30 μl reaction mix containing 5.3 mM dTTP, 25 mM Tris.HCL pH 7.5, 0.1 M sodium cacodylate, 1 mM $CoCl_2$, 0.1 M DTT and 170 U terminal deoxynucleotidyl transferase (Pharmacia). After one hour incubation, the reaction was stopped and the tailed probes were precipitated and washed with ice-cold ethanol. Probes were dissolved in 6x SSC at their respectively specific concentrations and applied as horizontal lines on membrane strips in concentrations between 0.2 and 2.5 pM/ml. Biotinylated DNA was applied alongside as positive control (LiPA line 1) . The oligonucleotides were fixed to the membrane by baking at 80°C for 12 hours. The membrane was than sliced into 4 mm strips. The design of this strip is indicated in Figure 2.

Example 3. LiPA test performance

Equal volumes (10 μl each) of the biotinylated PCR fragment and of the denaturation solution (DS; 400 mM NaOH/10 mM EDTA) were mixed in test

troughs and incubated at room temperature for 5 minutes. Then, 2 ml of the 37°C prewarmed hybridization solution (HS, 3x SSC/0.1% SDS) was added, followed by the addition of one strip per test trough. Hybridisation occured for 1 hour at 50 ± 0.5°C in a closed shaking water bath. The strips were washed twice with 2 ml of stringent wash solution (3x SSC/0.1% SDS) at room temperature for 20 seconds, and once at 50°C for 30 minutes. Following this stringent wash, strips were rinsed two times with 2 ml of the Innogenetics standard Rinse Solution (RS). Strips were incubated on a rotating platform with the alkaline phosphatase-labelled streptavidin conjugate, diluted in standard Conjugate Solution for 30 minutes at room temperature (20 to 25°C). Strips were than washed twice with 2 ml of RS and once with standard Substrate Buffer (SB), and the colour reaction was started by adding BCIP and NBT to the SB. After maximum 30 minutes at room temperature, the colour reaction was stopped by replacing the colour compounds by distilled water. Immediately after drying, the strips were interpreted. Reactivities were considered positive whenever the reactivity was stronger than the reaction on the negative control. Strips can be stored on a dry dark place. The complete procedure described above can also be replaced by the standardized Inno-LiPA automation device (*auto*-LiPA).

Example 4. *Selection of reference material.*

PCR fragments were prepared, derived from members of the different genotypes, the different preCore wild type and mutant sequences, drug resistant motifs and vaccine escape mutants. The PCR fragments were amplified with primers lacking the biotine group at their 5'-end and cloned into the pretreated EcoRV site of the pGEMT vector (Promega). Recombinant clones were selected after α-complementation and restriction fragment length analysis, and sequenced with plasmid primers. Other biotinylated fragments were directly sequenced with a dye-terminator protocol (Applied Biosystems) using the amplification primers. Alternatively, nested PCR was carried out with analogs of the primers, in which the biotine group was replaced with the T7- and SP6-primer sequence, respectively. These amplicons were than sequenced with an SP6- and T7-dye-prim r procedure.

By doing so, a reference panel of recombinant clones was prepared, which is necessary for optimizing LiPA probes.

Example 5: Genotyping HBV-infected serum samples.

Only after creating a sequence alignment as shown in Figure 1, it became clear which regions could be useful for HBV genotyping. The preS1 region seems to be suitable because of the high degree of variability. Probes were therefore designed to cover most of these variable regions as shown in Table 1. Only a limited selection of probes was retained because of their specific reaction with the reference panel. The most important ones are indicated as boxed regions in Figure 1. These selected probes were then applied in a LiPA format indicated in Figure 2, as line number 2 to 14. Some of the probes could be applied together in one line, because of their universal character, while others need to be applied separately. With the selection of probes thus obtained, serum samples collected in different parts of the world (Europe, South-America, Africa, Middle-East) were tested. The upper part of Figure 3 shows the reactivity of a selection of samples on these probes. Genotyping of these samples is straightforward, with samples 2 to 8 belonging to genotype A, samples 9 and 10 belonging to genotype B, samples 11 and 12 belonging to genotype C, samples 13 to 19 belonging to genotype D, samples 20 to 23 belonging to genotype E, and sample 24 belonging to genotype F.

Genotyping can also be performed in the HBsAg region. Again, probes were designed to cover most of the variable regions shown in Fig. 1. Only a limited selection of probes were retained. These probes are boxed in Fig.1 and are listed in Figure 4. A LiPA strip was prepared carrying these probes and samples belonging to the different genotypes were characterized, as shown in Fig. 5.

Example 6. Scanning the preCore region for mutations.

HBeAg expression can be regulated at the transcriptional and translational level. It is postulated that a transcriptional regulation exists due to the presence of a dinucleotide variation in the promoter region of the preCore mRNA. Probes

covering the wild type (e.g. probe HBPr 88) and the mutant (e.g. HBPr 89) motif were selected and their positions are indicated in the alignment shown in Figure 1, and applied on the LiPA strip as line 15 and 16 (Figure 2).

At the translational level, much more mutations might arise, all possibly resulting in abrogation of the HBeAg expression: any mutations at codon 1 (ATG) destroying translation initiation, codon 2 (CAA to TAA), codon 7 (TGC to TGA), codon 12 (TGT to TGA), codon 13 in genotype B, C, D, E, F (TCA to TGA or TAA), codon 14 (TGT to TGA), codon 18 (CAA to TAA), codon 21 (AAG to TAG), codon 23 (TGC to TGA), codon 26 (TGG to TAG or TGA), codon 28 (TGG to TAG or TGA). However, due to secondary contrain of the encapsidation signal, most of the mutations occur at codon 28 (TGG to TAG). Along with the mutation at codon 28, a second mutation at codon 29 (GGC to GAC) is often observed. In the case of genotype A and again as a consequence of the secondary constrain, stop codon mutations at codon 28 are only likely to occur after selection of a codon 15 mutation (CCC to CCT). Hence, correct interpretation of preCore mutations is genotype dependent. In addition to the above mentioned stop codons, a huge amount of different deletion- or insertion-mutations in the preCore open reading frame might give essentially the same result.

In order to develop a sensitive assay to detect the relevant mutations and the hypothetical mutations, a probe scanning procedure was developed. Partially overlapping probes were designed and applied in a LiPA format (Figure 2, line 17 to 27). In this assay format, wild type sequences over the complete preCore region, together with the codon 15 variation for genotype A versus non-A genotypes, and the most common mutations at codon 28 (TAG), at codon 29 (GAC) and the combination of codon 28 and 29 (TAGGAC) are positively recognized. Absence of reactivity at one of the other probes is always indicative for the presence of a variation. The exact nature of this variation can then be revealed by sequence analysis or with further designed LiPA probes.

Figure 3 shows the reactivity of the selected genotyped samples on the probes for the preCore region. Samples were previously tested for the presence of HBeAg or for anti-HBe. The interpretation of the reactivity on the LiPA probes for

each sample is indicated below each strip. This approach allowed for the simultaneous screening of a sample for preCore mutations and the characterization of the viral genotype.

Figure 6 also shows a panel of samples with mutations in the preCore region, as well as wild type samples. The probes used in this assay are listed in Figure 4. This assay includes a codon 29 mutant (M4 motif), which was not present in the experiment in Figure 3.

***Example** 7. Detection of mutants in the HBsAg region.*

Vaccine escape mutants have been described. The most commonly found mutant is the variation at codon 145 of HBsAg (G145R or GGA to AGA). LiPA probes are designed to detect wild type and mutant probes. Genotypic variations are present in the vicinity of codon 145. Therefore, genotype A is covered by probe 77, genotype B by probe 78, genotype C by probe 79, and genotype D/E by probe 80. Hence, in principle, it is possible to genotype and detect the wild type strains of the virus in one single experiment. Mutant target sequences are covered by probe 81 and 82 for genotype A and D, respectively. Probe 83 can be used as a positive control in these experiments. Further detection of mutants in the *a* determinant region is possible by means of a probe scanning approach. Herefore, probes are designed to cover the wild type sequence of the different genotypes over the HBsAg epitope region and applied in a LiPA format. Again here, absence of staining at one of these probes is indicative for the presence of a mutant strain. The exact nature of this variant is then determined by sequencing analysis.

***Example** 8. Detection of HBV strains resistant to lamivudine.*

Through analogy with HIV and the resistance against the anti-viral compound 3TC (lamivudine or (-)-ß-1-2',3'-dideoxy-3'-thiacytidine), it was predicted that upon treatment of HBV-infected patients with 3TC, viral strains would be selected showing resistance at the YMDD motif in the HB pol gene. The YMDD motif is physically located in the HBsAg region, but is ncoded in another reading frame. Hence, this part of the HBV pol region is amplified with the primer combination

HBPr 74-HBr 94, but not with the combination HBPr 74-HBr 76. Probes covering the wild type YMDD motif and YVDD mutant motif are indicated in Figure 1, respectively probes 95 to 100 and 101 to 103, as well as probes 115, 116, 127 and 132, the latter probes yielding the best results in the LiPA assay. Such an assay was used to determine the presence of mutations in the YMDD motif in serum of a HBV-infected patient during treatment with lamivudine. Fig. 7 shows that in the first phase of the treatment (May 1995) no mutations were detected. During the treatment, the viral load decreased, reaching a level of approximately 10^4 during November and December 1995, whereafter a breakthrough was observed, resulting in a level as high as during the first months of the treatment by June 1996 . Interestingly, a LiPA assay performed in February 1996 indicated that the majority of virus present, possessed a mutation in the YMDD motif, which had changed to YVDD. In June 1996, no more wild type motif, but only mutant YVDD could be detected. With this assay, resistant HBV strains can thus easily be detected. Furthermore, the combined detection of the YMDD motif and preCore mutants might be clinically important in prediction and prognosis of further treatment.

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CLAIMS

1. Method for detection and/or genetic analysis of HBV in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in said sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with a combination of at least two nucleotide probes, with said combination hybridizing specifically to a mutant target sequence chosen from the HBV RT pol gene region and/or to a mutant target sequence chosen from the HBV preCore region and/or to a mutant target sequence chosen from the HBsAg region of HBV and/or to a HBV genotype-specific target sequence, with said target sequences being chosen from Figure 1, and with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV genotype and/or mutants present in said sample from the differential hybridization signal(s) obtained in step (iv).

2. Method according to claim 1, characterized further in that in step (iii) a combination of at least two oligonucleotide probes is used and that said combination of probes hybridizes specifically to at least two of the following groups of target sequences:

a mutant target sequence chosen from the HBV RT pol gene region,

a mutant target sequence chosen from the HBV preCore region,

a mutant target sequence chosen from the HBsAg region of HBV,
a HBV genotype-specific target sequence.

3. Method according to claim 1, characterized further in that in step (iii) a combination of at least three oligonucleotide probes is used and that said combination of probes hybridizes specifically to at least three of the following groups of target sequences:
a mutant target sequence chosen from the HBV RT pol gene region,
a mutant target sequence chosen from the HBV preCore region,
a mutant target sequence chosen from the HBsAg region of HBV,
a HBV genotype-specific target sequence.

4. Method according to claim 1, characterized further in that in step (iii) a combination of at least four oligonucleotide probes is used and that said combination of probes hybridizes specifically to all of the following groups of target sequences:
a mutant target sequence chosen from the HBV RT pol gene region,
a mutant target sequence chosen from the HBV preCore region,
a mutant target sequence chosen from the HBsAg region of HBV,
a HBV genotype-specific target sequence.

5. Method according to any of claims 1 to 4, characterized further in that the oligonucleotide probes used in step (iii) are selected from Table 1, wherein:
-the probes hybridizing specifically to mutant target sequences chosen from the RT pol region of HBV are selected from the following list:
SEQ ID 114, SEQ ID NO 115, SEQ ID NO 116, SEQ ID NO 117, SEQ ID NO 127, SEQ ID NO 128, SEQ ID NO 129, SEQ ID NO 130, SEQ ID NO 131, SEQ ID NO 132, SEQ ID NO 133, SEQ ID NO 227, SEQ ID NO 228, SEQ ID NO 229, SEQ ID NO 230, SEQ ID NO 231, SEQ ID NO 232, SEQ ID NO 233, SEQ ID NO 234, SEQ ID NO 243, SEQ ID NO 244, SEQ ID NO 245, SEQ ID NO 246, SEQ ID NO 247, SEQ ID NO 248, SEQ ID NO 249, SEQ ID

NO 250, SEQ ID NO 251, SEQ ID NO 252, SEQ ID NO 253, SEQ ID NO 254, SEQ ID NO 255, SEQ ID NO 256, SEQ ID NO 257, SEQ ID NO 258, SEQ ID NO 259, SEQ ID NO 260, SEQ ID NO 261, SEQ ID NO 262, SEQ ID NO 263, SEQ ID NO 264, SEQ ID NO 265, SEQ ID NO 266, SEQ ID NO 267, SEQ ID NO 268, SEQ ID NO 269, SEQ ID NO 270, SEQ ID NO 271, SEQ ID NO 272, SEQ ID NO 273, SEQ ID NO 274, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278, and/or

-the probes hybridizing specifically to mutant target sequences chosen from the preCore region of HBV are selected from the following list:
SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 42, SEQ ID NO 43, SEQ ID NO 44, SEQ ID NO 45, SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, SEQ ID NO 66, SEQ ID NO 67, SEQ ID NO 68, SEQ ID NO 88, SEQ ID NO 89, SEQ ID NO 90, SEQ ID NO 91, SEQ ID NO 118, SEQ ID NO 119, SEQ ID NO 120, SEQ ID NO 121, SEQ ID NO 122, SEQ ID NO 123, SEQ ID NO 124, SEQ ID NO 125, SEQ ID NO 126, and/or

-the probes hybridizing specifically to mutant target sequences chosen from the HBsAg region of HBV are selected from the following list:
SEQ ID NO 77, SEQ ID NO 78, SEQ ID NO 79, SEQ ID NO 80, SEQ ID NO 81, SEQ ID NO 82, SEQ ID NO 136, SEQ ID NO 137, SEQ ID NO 138, and/or

-the probes hybridizing specifically to genotype-specific target sequences of HBV are selected from the following list:
SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32,

SEQ ID NO 33, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, SEQ ID NO 53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO'57, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 60, SEQ ID NO 61, SEQ ID NO 62, SEQ ID NO 63, SEQ ID NO 64, SEQ ID NO 65, SEQ ID NO 73, SEQ ID NO 74, SEQ ID NO 92, SEQ ID NO 93, SEQ ID NO 77, SEQ ID NO 78, SEQ ID NO 79, SEQ ID NO 80, SEQ ID NO 81, SEQ ID NO 82, SEQ ID NO 139, SEQ ID NO 140, SEQ ID NO 141, SEQ ID NO 142, SEQ ID NO 143, SEQ ID NO 144, SEQ ID NO 145, SEQ ID NO 146, SEQ ID NO 147, SEQ ID NO 148, SEQ ID NO 149, SEQ ID NO 150, SEQ ID NO 151, SEQ ID NO 152, SEQ ID NO 153, SEQ ID NO 154, SEQ ID NO 155, SEQ ID NO 156, SEQ ID NO 157, SEQ ID NO 158, SEQ ID NO 159, SEQ ID NO 160, SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169, SEQ ID NO 170, SEQ ID NO 171, SEQ ID NO 172, SEQ ID NO 173, SEQ ID NO 174, SEQ ID NO 175, SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO 183, SEQ ID NO 184, SEQ ID NO 185, SEQ ID NO 186, SEQ ID NO 187, SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194.

6. Method according to any of claims 1 to 5, wherein the oligonucleotide probes of step (iii) are characterized in that they specifically hybridize to target sequences in the RT pol region of HBV and permit detection of mutations that confer resistance to lamivudine, with said probes being for instance SEQ ID NO 114, SEQ ID NO 115, SEQ ID NO 116, SEQ ID NO 117, SEQ ID NO 127, SEQ ID NO 128, SEQ ID NO 129, SEQ ID NO 130, SEQ ID NO 131, SEQ ID NO 132, SEQ ID NO 133, SEQ ID NO 227, SEQ ID NO 228, SEQ ID NO 229, SEQ ID NO 230, SEQ ID NO 231, SEQ ID NO 232, SEQ ID NO 233, SEQ ID NO 234, SEQ ID NO 243, SEQ ID NO 244, SEQ ID NO 245, SEQ ID NO 246, SEQ ID NO 247, SEQ ID NO 248, SEQ ID NO 249, SEQ ID NO 250, SEQ ID NO 251, SEQ ID NO 252, SEQ ID NO 253,

SEQ ID NO 254, SEQ ID NO 255, SEQ ID NO 256, SEQ ID NO 257, SEQ ID NO 258, SEQ ID NO 259, SEQ ID NO 260, SEQ ID NO 261, SEQ ID NO 262, SEQ ID NO 263, SEQ ID NO 264, SEQ ID NO 265, SEQ ID NO 266, SEQ ID NO 269, SEQ ID NO 270, SEQ ID NO 271, SEQ ID NO 272, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278.

7. Method according to any of claims 1 to 5, wherein the oligonucleotide probes of step (iii) are characterized in that they specifically hybridize to target sequences in the RT pol region of HBV and permit detection of mutations that confer resistance to penciclovir, with said probes being for instance SEQ ID NO 267, SEQ ID NO 268, SEQ ID NO 269, SEQ ID NO 270, SEQ ID NO 271, SEQ ID NO 272, SEQ ID NO 273, SEQ ID NO 274, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278.

8. Method according to any of claims 1 to 5, wherein the oligonucleotide probes of step (iii) are characterized in that they specifically hybridize to target sequences in the RT pol region of HBV and permit detection of at least one of the mutations that give rise to a change of the following amino acids: F at position 514, V at position 521, P at position 525, L at position 527, M at position 552, V at position 555, with said probes being for instance SEQ ID NO 114, SEQ ID NO 115, SEQ ID NO 116, SEQ ID NO 117, SEQ ID NO 127, SEQ ID NO 128, SEQ ID NO 129, SEQ ID NO 130, SEQ ID NO 131, SEQ ID NO 132, SEQ ID NO 133, SEQ ID NO 227, SEQ ID NO 228, SEQ ID NO 229, SEQ ID NO 230, SEQ ID NO 231, SEQ ID NO 232, SEQ ID NO 233, SEQ ID NO 234, SEQ ID NO 243, SEQ ID NO 244, SEQ ID NO 245, SEQ ID NO 246, SEQ ID NO 247, SEQ ID NO 248, SEQ ID NO 249, SEQ ID NO 250, SEQ ID NO 251, SEQ ID NO 252, SEQ ID NO 253, SEQ ID NO 254, SEQ ID NO 255, SEQ ID NO 256, SEQ ID NO 257, SEQ ID NO 258, SEQ ID NO 259, SEQ ID NO 260, SEQ ID NO 261, SEQ ID NO 262, SEQ ID NO 263, SEQ ID NO 264, SEQ ID NO 265, SEQ ID NO 266, SEQ ID NO 267, SEQ ID NO 268, SEQ ID NO 269, SEQ ID NO 270, SEQ ID

NO 271, SEQ ID NO 272, SEQ ID NO 273, SEQ ID NO 274, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278.

9. Method according to any of claims 1 to 5, characterized further in that the probes of step (iii) hybridize specifically to a genotype-specific target sequence from the HBsAg region, said probes being for instance SEQ ID NO 77, SEQ ID NO 78, SEQ ID NO 79, SEQ ID NO 80, SEQ ID NO 81, SEQ ID NO 82, SEQ ID NO 139, SEQ ID NO 140, SEQ ID NO 141, SEQ ID NO 142, SEQ ID NO 143, SEQ ID NO 144, SEQ ID NO 145, SEQ ID NO 146, SEQ ID NO 147, SEQ ID NO 148, SEQ ID NO 149, SEQ ID NO 150, SEQ ID NO 151, SEQ ID NO 152, SEQ ID NO 153, SEQ ID NO 154, SEQ ID NO 155, SEQ ID NO 156, SEQ ID NO 157, SEQ ID NO 158, SEQ ID NO 159, SEQ ID NO 160, SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169, SEQ ID NO 170, SEQ ID NO 171, SEQ ID NO 172, SEQ ID NO 173, SEQ ID NO 174, SEQ ID NO 175, SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO 183, SEQ ID NO 184, SEQ ID NO 185, SEQ ID NO 186, SEQ ID NO 187, SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 214, SEQ ID NO 215, SEQ ID NO 216, SEQ ID NO 217, SEQ ID NO 218, SEQ ID NO 219.

10. Method according to any of claims 1 to 5, characterized further in that the probes of step (iii) hybridize specifically to a genotype-specific target sequence from the preS1 region, said probes being for instance SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, SEQ ID NO 53, SEQ ID

NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 60, SEQ ID NO 61, SEQ ID NO 62, SEQ ID NO 63, SEQ ID NO 64, SEQ ID NO 65, SEQ ID NO 73, SEQ ID NO 74, SEQ ID NO 92, SEQ ID NO 93.

11. A composition comprising at least one probe as defined in any of claims 1 to 10.

12. A composition comprising at least one probe as defined in claim 5.

13. Use of a composition of probes as defined in claims 11 and/or 12 for *in vitro* diagnosing and/or monitoring HBV mutants and/or genotypes present in a biological sample.

14. Assay kit for the detection and/or the genetic analysis of HBV mutants and/or genotypes present in a biological sample according to the method of any of claims 1 to 10, comprising the following components :

(i) when appropriate, a means for releasing, isolating or concentrating the polynucleic acids present in said sample;

(ii) when appropriate, at least one suitable primer pair;

(iii) at least one of the probes according to claim 11 and/or 12, possibly fixed to a solid support;

(iv) a hybridization buffer, or components necessary for producing said buffer;

(v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

(vii) when appropriate, a means for attaching said probe to a known location on a solid support.

Figure 1

```
                                                                                HBPr 4
            1
HBVXCPS     AATTCCACTGCCTTCCACCAAGCTCTGCAGGATCCCAAAGTCAGGGGTCTGTATCTTCCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGCTCCGAATATTGCCTCTCACATCTCGTCAATCTCCGCGAGGACTGGGGACCCTGTG
HBVADW      --------------G-----------------------G-----------------------------------------------------------------------------------------------------------------
HVHEPB      ------------------------------A--C----G----------------T-----------------------------------------------------------------------------C------------
S50225      --------------------------------------G-----------------------------------------------------------------------------------------------------------------
HPBADW2CG   ---------A--T-----------------A-------G---------C------T--------------------------C-C-------T---A-C----------------------------T------T-----------CA
HPBADW3     --C------CA-G---------A----T--A-------G--------C------CT---------------------------------T--A---C-C-------TC---A----------TAT---C----------------
HPBADWZ     --C------CA-G---------A----T--A-------G--------C------CT---------------------------------T--A---C-C--T----TC---A----------TAT---A----------------
HPBADW1     --C------CA-T---------A----T--A-------G--------C------CT--------------------------------G--------A-----C--T---AGC---A----------TAT---A----------------
HPBADW2     --C------CA-T---------A----T--A------GG--------CC-----CT------------------------------G-G--------A-----C--T----GC---A----------TAT---A---------------A
HPBCGADR    --C------CA-A--T-------TC----TA-------G---G-----C--A---T----C-----------------C---------------T-G---C--C------A-C---A------------T------------------CA
HBVADRM     --C------CA-A--T-------TC----TA-------G---G-----C--A---T----C-----------------C---------------T-----C--C------A-C---A------------T------------------CA
HPBADRA     --C------AA-A----------------TA-------G---G-----C--A---T------------------------C---------------T-----C--C--T---A-C---A----------T-T------------------CA
HPBCG       --C------AA-A----------------TA-------G---G-----C--A---T------------------------C---------------T-----C--C------A-C---A----------T-T------------------CA
HEHBVAYR    --C------AA-A----------------TA-------G---G-----C--A---T------------------------C---------------T-----C--C------A-C---A----------T-T------------------CA
HBVADR      ---------AA-A----------------TA-------G---G-----C--A---T------------------------C---------------T-----C--C------A-C---A----------T-T------------------CA
HBVADR4     --C------AA-A----------------TA--C----G---G-----C--A--CT------------------------C---------------T-----C--C------A-C---A------------T------------------CA
HPBADR1C    --C------AA-A----------------TA--C----G---G-----C--A--CT------------------------C---------------T-----C--C------A-C---A----------T-T------------------CA
HPBADRC     --C------AA-A------------G---TA-------G---G-----C--A----------------------------C-------G-------T-----C--C------A-C---A----------T-T------------------CA
HBVPREX     --C------AA-A----------------TA-----A-G---G-----C--C---T-C----------------------C---------------T-----C--C------A-C---A----------T-T------------------CA
HPBETNC     --C------CA-A----------------TA-------G---G-----C--A---T------------------------C---------------T-----C--C------A-C---A----------T-T------------------C-
HHVBC       --C------AA-A-----A-------------------G--------TC--T---T------------------------C---------------T-----C--C--------T---T----------T-T-------T-----------A
HHVCCHA     --C------AA-A-----A----------TA-------G---G-----C--A---T------------------------C---------------T-----C--C--------T---T----------T-T-------------------A
HBVAYWMCG   ---------AA-----------A----A--A-----/////////////////////C-------------------------------------T-----C--C--T---------A----------T-A-------T----------CA
HBVAYWC     --C------AA-----------A-------A-------G---G--A--C------T-C-------------------------------------T-----C--C----------T-A----------T-T-------T----------C-
HBVAYWCI    --C------AA-T---------A-------A-------G---G--A--C--A---T-C-------------------------------------T-----C--C--------C-T-A----------T-T-------T------------
HBVAYWE     --C------AA-----------A-------A-------G---G--A--C------T-C-------------------------------------T--T--C--C--------C-T-A-------------------------------CA
HBVDNA      --C------AA-----------A-------A-----A-G---G--A--C------T-C-------------------------------------T--T--C--C--------C-T-A---------------------------------
HPBHBVAA    --C------AA-----------A-------A-------G---G--A--C--------C-------------------------------------T-----C--C--T-----C---A----------T-T-------T----------C-
XXHEPAV     --C------AA-----------A-------A-------G---G--A--C------T-C-------------------------------------T-----C--C--T-----C---A----------T-T-------T----------C-
HBVORFS     --C------AA-T---------A-------A-------GG--G--A--C------T-C-------------------------------------T-----C--C--------C---A----------T-T-------T----------CA
HHVBE4      --C------AA-A--T--T-------------------G---A--A--C------T------------------------C-------G-------T-----C--C------A-T--------------T-T-------T----------CA
HHVBBAS     ---------AA-A-------------------------G---A--A--C------T------------------------C-------G-------T-----C--C------A-T--------------T-T-------T----------CA
HHVBF       --C--A---CA-----------------TT--------GG--A-----CA-----T-------------------------------CAG-------------C------------T--CA--A---------T---A--------G----CT
HHVBFFOU    --C--A---CA----------G------TT------G-GG--A-----CA-----T---------------------------C-GCAG-------------C------------T--CA--A---------T---A--------G----CT
HBVADW4A    --C--A--CCAG----------------TT--------GG--A-----C-----CT-C-------------------------G--CAG-------------C------------T--CA--A-------T-T---A--------G----CT
```

```
                  Start HBsAg
HBVXCPS    ACGAAC|ATGGAGAACATCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGGTCACCCGTGTGT
HBVADW     ----T-|-----------------------------------------------------------------------------------------------------------------------------------A------------
HVHEPB     ------|-------------------------------------C-------------------------------------------------------------------------------------------------A------------
S50225     ------|----------------------------------------------------------C------------------------------------------------------------------------------------C
HPBADW2CG  C-----|-----------------------------------------------------------------------------------------------------------------------------------AG-----------
HPBADW3    C-----|------------G--------C---------------------------------------C--------A--------------T-T------------------------------------------AAA-----------
HPBADWZ    C-----|------------G--------C------------------------------------------------A--------------A---------------------------------------------AA-----------
HPBADW1    C-----|------------G--------C------------------------------------------------A--------------A---------------------------------------------AA-----------
HPBADW2    C-----|------------G--------C------------------------------------------------A--------------A---------------------------------------------AA-----------
HPBCGADR   C-----|-------G--CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HBVADRM    C-----|-------G--CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HPBADRA    C-----|-------G--CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HPBCG      C-----|-------G--CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HEHBVAYR   C-----|-------G--CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HBVADR     C-----|----------CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HBVADR4    C-----|----------CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HPBADR1C   C-----|----------CA-------------------------------------------------------------------------A----------C---------------------------------CAG-----AC----
HPBADRC    C-----|----------CA-------------------------------------------------------------------------A---------------------------------------------AG-----AC----
HBVPREX    C-----|-------G--CA-------------------------------------------------------------------------A--------------------T------------------------AA-----AA----
HPBETNC    C-----|----------CA-------------------------------------------------------------------------A---------------///---------------------------AG-----AC----
HHVBC      ------|----------CA----------------------------------------------------------A--------------A---------------------------------------------AG-----------
HHVCCHA    ------|----------CA-------------------------------------------------------------------------A----------------G---------------------------AAG----AAG----
HBVAYWMCG  CT----|-----------------------------------------------------------------------------------------------------------------------------------AA-TA--------
HBVAYWC    CT----|-----------------------------------T-----------------------------------------------------------------------------------------------AA-TA--------
HBVAYWCI   -----T|----------------------------------------------------------------------------------------------------------------------------G------AA-TA--------
HBVAYWE    CT----|-----------------------------------------------------------------------------------------------------------------------------------AA-TA--------
HBVDNA     ----T-|---------------------------------------------G-------------------------------------------------------------------------------------AA-TA--------
HPBHBVAA   CT----|----------------------------------------------------------------------A---------------A--------------------------------------------AA-CA--------
XXHEPAV    CT----|-----------------------------------------------------------------------------------------------------------------------------------AA-TA--------
HBVORFS    CT----|-----------------------------------------------------------------------------------------------------------------------------------AA-CA--------
HHVBE4     C-----|-----A-G--------------------------------------------------------------A------------------------------------------------------------AG-T---------
HHVBBAS    C-----|-----A-G--------------------------------------------------------------A------------------------------------------------------------AG-T---------
HHVBF      -T----|---------------------C---------------G--------------T--G--------------A--------------A---------------------------------------------ACT----AG----
HHVBFFOU   -T----|-----C---------------C------------------------------T--G--------------A--------------A---------------------------------------------ACT-----G----
HBVADW4A   -T----|-----C-----T---------C------------------------------T--G--------------A--------------A---------------------------------------------ACT-----G----
```

```
            301
HBVXCPS     CTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTG
HBVADW      -----------------------------------------------------------------------------------------------------------------------------------------A------------
HVHEPB      ----------------GA-----------------------------------------------------------------------------------------------------------------------A------------
S50225      -----------------------------------------------------------------------------------------------------------------------------------------A------------
HPBADW2CG   -----------------------------------------------------------------------------------------------------------------------------------------A------------
HPBADW3     ----------------------A--T-----G------------T-GT--------G--------------------------------------------C-------G----------------------------------------
HPBADWZ     ----------------------A--T-----G------------T-GT--------G--------------------------------------------C-------G----------------------------------------
HPBADW1     ----------------------A--T-----G--------------GT-----------------------------------------------------C-------G----------------------------------------
HPBADW2     ----------------------A--T-----G--------------GT-----------------------------------------------------C-------G----------------------------------------
HPBCGADR    -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVADRM     -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HPBADRA     -C---------------------------------------------T------------------------------------------------------------------------------------------------------
HPBCG       -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HEHBVAYR    -C---------------------------------------------T----------C---------C---------------------------------------------------------------------------------
HBVADR      -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVADR4     -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HPBADR1C    -----------------------------------------------T--------------------------T---------------------------------------------------------------------------
HPBADRC     -C-----C---------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVPREX     -C---------------------------------------------T----------C---------C---------------------------------------------------------------------------------
HPBEINC     -C---------------------------------------------T--------------------C-------------------------------------------------------------------------C-------
HHVBC       -C---------------------------------------------T--------------------C--------------------------------C------------------------------------------------
HHVCCHA     -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVAYWMCG   ----------------------------------------------------------C------------------------------------------C------------------------------------------------
HBVAYWC     ----------------------------------------------------------C------------------------------------------C------------------------------------------------
HBVAYWCI    ----------------------------------------------------------C-------------------------------G----------C------------------------------------------------
HBVAYWE     -----------------------------------------------T----------C------------------------------------------C------------------------------------------------
HBVDNA      ------------------------------C-----------------------------------------------------------------------------------------------------------------------
HPBHBVAA    ----------------------------------------------------------C------------------------------------------C------------------------------------------------
XXHEPAV     ----------------------------------------------------------C------------------------------------------C------------------------------------------------
HBVORFS     ----------------------------------------------------------C------------------------------------------C-----------------T--------------------------A---
HHVBE4      -----------------------------------------------T--------------------C--------------------------------C------------------------------------------------
HHVBBAS     -------------------------------G---------------T--------------------C--------------------------------C------------------------------------------------
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HBVADW4A    -C-----------------------------------T--------------------C---------C-----T--------------------------C------------------------------------------------
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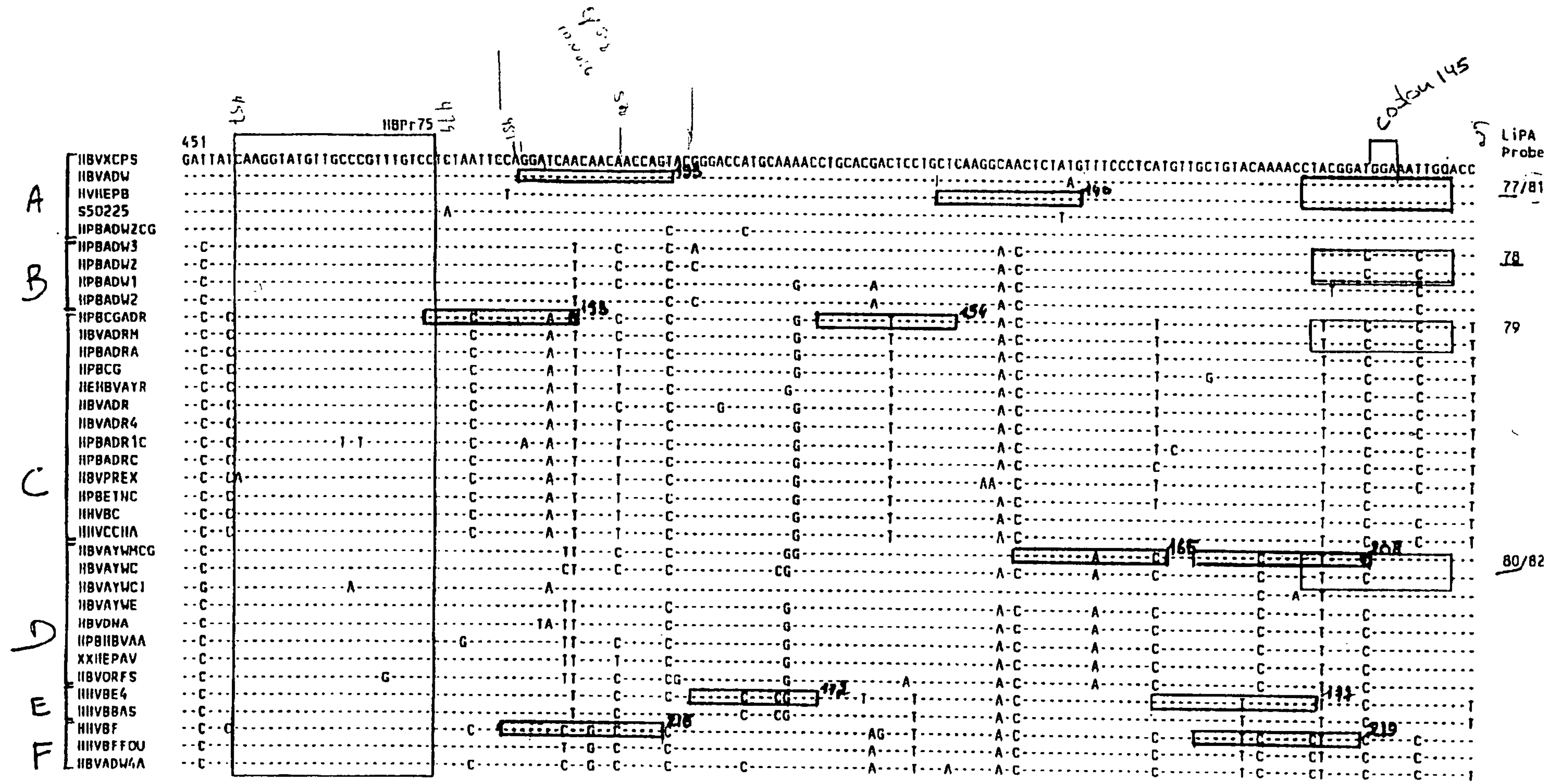
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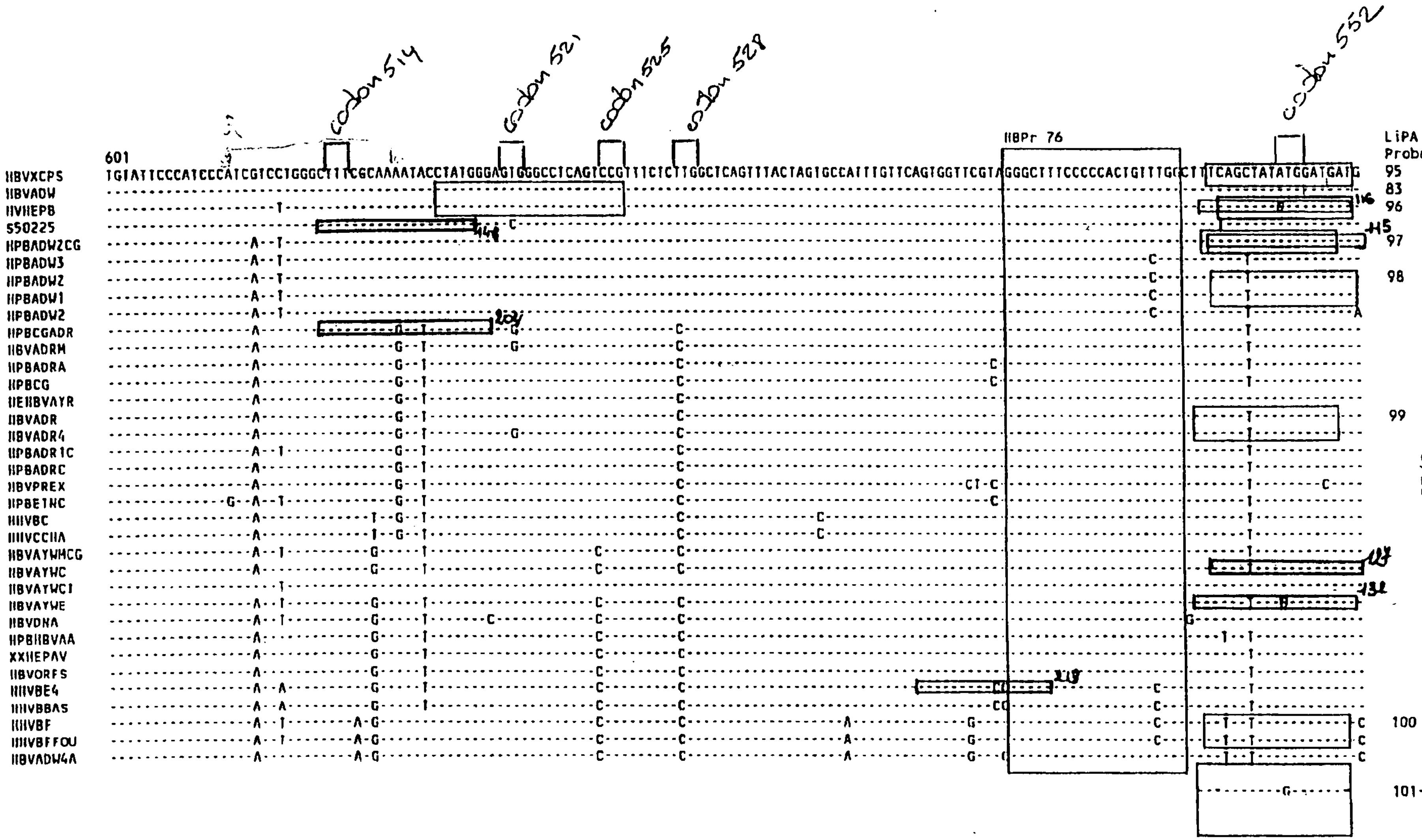
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HVHEPB
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HPBADW3
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HPBCG
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HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWCI
HBVAYWE
HBVDNA
HPBHBVAA
XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A
95
83
96
97
98
99
100
101-10
116
115
144
204
123
132
219

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                                                         HBPr94                                          End of HBsAg
           751
HBVXCPS    TGGTATTGGGGGCCAAGACTGTACAGCATCGTGAGTCCCTTTATACCGCTGTTACCAATTTTCTTTTGTCTCTGGGTATACATTTAA ACCCTAACAAAACAAAAAGATGGGGTTATTCCCTAAACTTCATGGTCTACATAATTGGAAGTT
HBVADW     ------------------T-------------------------------------------------------------------- ---------------------------------------------G-----------------
HVHEPB     ------------------T------------------T------------------------------------------------- ---------------------------------------------GT--TG------------
S50225     ------------------T-----------------G-------------------------------------------------- ---------------------------------------------GT---G------------
HPBADW2CG  -----C------------T-------A----T----------------------------------------T-------------- ----------------G-----------------G--T-------GT--TG------------
HPBADW3    ----T-------------T-------A----T-------------G--------------------------T-------------- -----C-G-----------------C--C-----T----------GG--TG------------
HPBADW2    ----T-------------T-------A----T-------------G--------------------------T-------------- -----C-G-----------------C--C-----C----------GG--TG------------
HPBADW1    ------------------T-------A----T-------------G--------------------------T-------------- -------------------------A--------T----------GA--TG-------G----
HPBADW2    ----T-------------T-------A----T-------------G--------------------------T-------------- -----C-------------------A--------T----------GA--TG-------G--C-
HPBCGADR   ------------------T-------A----T-----------T----T--A--------------------T------------G- -------T-----C---C-T-----C--C-----T----------GA--TG-------C----
HBVADRM    ------------------T-------A----T-----------T----T--A--------------------T------------G- -------T-----C---C-T-----C--C-----T----------GA--TG-------C----
HPBADRA    ------------------T-------A----T-----------T----T--A--------------------T------------G- -------T-----C---C-T-----C--C-----T----------GA--TG------------
HPBCG      ------------------T-------A----T-----------T----T--A--------------------T------------G- -------T-----C---C-T-----C--C-----T----------GA--TG------------
HEHBVAYR   ------------------T-------A----T-----------T----T--A--------------------T------------G- -------T-----C---C-T-----C--C-----T----------GA--TG------------
HBVADR     ------------------T-------A----T-----------T----T--A--------------------T-------------- -------T-----C---C-T-----C--C-----T----------GA--TG------------
HBVADR4    ------------------T-------A----T-----------T----T--A--------------------T-------------- -------T-----C---C-T-----C--C-----T----------GA--TG--------T---
HPBADR1C   ------------------T-------A----T-----------T----T--A--------------------T------------G- ----C--T-----C---C-T-----C--------T--T-------GA--TG--------T---
HPBADRC    ------------------T-------A----T-----------T----T--A--------------------T------------G- -----C-T-----C---C-T-----C--C-----T----------GA--TG------------
HBVPREX    ------------------T-------A----T-----------T----T--A----------A-G-------T---C--------G- -------T-----C--GC-T-----C--C-----T----------GA--TG------------
HPBETNC    ------------------T-------A----T----------------T--A-------------G------T------------G- -------T-----C---C-T-----C--C-----T----------GA--TG------------
HHVBC      ------------------T-------A----T----------------T--A--------------------T-------------- -------T-----C-----------C--------T----------G---TG------------
HHVCCHA    ------------------T-------A----T----------------T--A--------------------T------------G- -------T--G--C-----------C--------T----------G---TG------------
HBVAYWMCG  ------------------T------------T-----------T----------------------------T---C---------- ----------------------------C--TT--C---------G---TG-C------T---
HBVAYWC    ------------------T------------T-----------T----------------------------T-------------- ----------------G-----------C--T-----T--T----G---TG-C------T---
HBVAYWC1   ------------------T------------T-----------T----------------------------T-------------- ----------------G-----------C--TT--C-T-------G---TG-C------T---
HBVAYWE    ------------------T------------T-----------T------------------------------------------- ---------------------------------------------GT----------------
HBVDNA     ------------------T-------------------------------------------------------------------- ---------------------------------------------GT----------------
HPBHBVAA   ----------------A-----T--C-----T-----------T----------------------------T----------C--- ----------------------------C--TT--C-T--T----G---TG-C------T---
XXHEPAV    ------------------T------------T-----------T---------------------C------T-------------- ----------------------------C--TT--C-T-------G---TG-C------T---
HBVORFS    ------------------T------------T-----------T----------------------------T-------------- -------------T--G-----------C--TT--C-T-------G---TG-C----------
HHVBE4     ------------------T------GA---------------------T-----------------------T-------------- -T--C--------------------A-----------T-------GT--TG------------
HHVBBAS    ------------------T-------A----T----------------------------------------T-------------- -T--C--------------------C--------T--T-------GT--TG------------
HHVBF      ----------[illegible]---T--------------------------------G--A---G-------C------- -TA--GCT--------------------CAA----C-T-------GT--TG-T-----T----
HHVBFFDU   ----------A-----AT----G--------T--------------------------------G--A---G-------C-----G- -TA-CTCT--------------------CAATT--C-T-------GT--TG-C-----C----
HBVADW4A   ----------------AT----G--------T--------------------------------G--A---G-------C------- -TA-CTCT--------------------C------C-T--T----GT--TG-C-----T----
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HBVXCPS    GGGGAACATTGCCACAGGATCATATTGTACAAAAGATCAAACACTGTTTTAGAAAACTTCCTGTTAACAGGCCTATTGATTGGAAAGTATGTCAAAGAATTGTGGGTCTTTTGGGCTTTGCTGCTCCATTTACTCAATGTGGATATCCTG
HBVADW     -------T-----------------------------------------------------------------------------------------------------------------------------A----------------
HVHEPD     ----------------------------------A--------------------------------------------------------------------------------------------T-----A----------------
S50225     ----------------------C---------------------------------------------------------------G-----------------------------------------T-----A----------------
HPBADW2CG  ----T-----------------------------A--------------------------------TC-A-----------------------G--C-----A--------A--------C-----------A--------T--C----
HPBADW3    ----G--C--A--C--A--A--------GTTG--A--------A--------G-----------A------------------------------C-------------------A-----------T--C--A----------------
HPBADWZ    ----C--C--A--C--A--A--------GTTG--AC-------A--C-----------------A-----A--------------G--G------C-------------------A-----------T--C--A--------T-------
HPBADW1    ----C----G------------------------CT------TA--------------------A-----------------------T------C-------------------G--------C--T-----G----------------
HPBADW2    ----C--------------A--------------A------ATG--G-----G-----------A------------------------------C-------------------G-----C--C--T--C--G----------------
HPBCGADR   ----T--T--A--G--A--A----------T---A-----G--A------C-G-----G-----A--T--A--------C--------------------------------------------C--T-----A--------C-------
HBVADRM    ----T--T--A--G--A--A----------T---A-----G--A------C-G-----G-----A--T--A--------C--------------------------------------------C--T-----A--------C-------
HPBADRA    ----T--T--A--------A---------TT---AC----G--A------C-------G-----A--T--A-----------------------------------------------------C--T-----A--------C-------
HPBCG      ----T--T--A--------A---------TT---AC----G--A------C-G-----G-----A--T--A-----------------------------------------------------C--T-----A--------C-------
HEHBVAYR   ----T--T--A--G-----A--------------AC----G--A------C-----T-G-----A--T--A-----------------------------------------------------C--T-----A--------C-------
HBVADR     ----T--T--A--G-----A----------T---AC----G--A------C-----T-G-----A--T--C-----------------------------------------------------C--T-----A-----C--C-------
HBVADR4    ----T--T--A--G--A--A----------T---A-----G--A------C-------G-----A--T--A-----------------------G---C-------------------------C--T-----A--------C-------
HPBADR1C   ----T--T--A--G--A--A----------T---A-----G--A------C-------G-----A--T--A-----------------------G-----------------------------C--T-----A--------C-------
HPBADRC    ----T--T--A--------A----------T---A-----G--A------C-G--G--G-----A--T--A--------------------------G--------------------------C--T-----A--------C-------
HBVPREX    ----T--T--A-----A--A--------------AC----G--A------C-------G-----C--T--A-----------------------G-----------------A-----------C--T-----A--------C-------
HPBETNC    ----T--G--A--------A--------------A-----G--A------C-G-----G-----A--T--A-----------------------------------------------------C--T-----A--------T-------
HHVBC      ----T--C--A-----A--A----------T---A----C---A------C----------------T--------------------G-----------------------------------C--T-----A--------G-------
HHVCCHA    ----T--C--A-----A--A----------T---A--------A------C-------------A--T-----------------G--C--C-----------------------A--------C--T-----A----------------
HBVAYWMCG  AT--GT----------A-----C--CAG---G--A------G-A-------------------------------------------GC------C-----------T-A-----T--------C--T-----A--------T-------
HBVAYWC    AT--GT-C--------A--A--C--CA-------A------G-A---------------------------------------------------C-------------------T--------C--T--------------T-------
HBVAYWCI   AT--GT-C--------A--A--C--CA-------A------G-A------------G--------------------------------------C----------------A--T--------C--T--C--A--------T-------
HBVAYWE    -------G--------A------------------------G-A-----------------------------------------------G---C-------------------------------------A----------------
HBVDHA     -------G-----------------------------------------------------------------------------------G---C-------------------T-----------------A--------T-------
HPBHBVAA   AT--GT-T--------A-----C--CA----G--A------G-A--------------------------------------------C------C-T-----------------A-----------T-----A--------T-------
XXHEPAV    AT--GT----------A-----C--CA----G--A------G-A--C-----------------------------------------C------C-T-----------------T--------C--T-----A--------T-------
HBVORFS    AT--GT----------A-----C--CA----G--A------G-A------------------A-------------------------C------C-T-----------------T--------C--T-----A--------T-------
HHVBE4     ----GT----A--------A--C--CA-------A---------------G-------C---------C--------------------------G----------------------------C--T-----A--------G-------
HHVBBAS    ----CT----A--------A--C--CA-------A------G----------------C---------C-------------------------------------------------------C--C-----A----------------
HHVBF      -------G--A--C--A--------------C--A------G-T------C-----G-------A--TC-C--A--------------T--------T-----------------------G--C--T-----C--------T-------
HHVBFFOU   -----G----A--C--A--------------C--A------G-A------C-----G-------A--TC-T--A-----C--------T------C-T--------A--------------------T-----C--------T-------
HBVADW4A   -----T----A--C--A-----C--------C--A-----GG-A--C---C-------G-----A--TC-T--A--------------T------C-C--A-----------------------C--T--C--C-----C--T-------
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HBVXCPS    CCTTAATGCCTTTGTATGCATGTATACAAGCTAAACAGGCTTTCACTTTCTCGCCAACTTACAAGGCCTTTCTAAGTAAACAGTACATGAACCTTTACCCCGTTGCTCGGCAACGGCCTGGTCTGTGCCAAGTGTTTGCTGACGCAACCC
HBVADW     ------------------------------------------------------------------------------------------------------------------------------------------------------
HVHEPB     ----------C---------------------------------------------------------------------------------------------------------------------------------------
S50225     ----------------------------------------------T-------------------------------------------C---------------------------------------------A---------T-------
HPBADWZCG  -A-----------------------------G-----------T-----------------------------------------T--------------------C-------------------------------------------
HPBADW3    -T-----------A-----------------------------T-----T--------G--T-------------AC------A--TC------------------------------A--------------------------------
HPBADWZ    -T-----------A-----------------------------T-----T--------G--T-------------CC------A--TC------------------------------A--------------------------------
HPBADW1    -T-----------A-----------------A-----------T-----------------------------C-----------T----C-----------------------------------------------------------
HPBADW2    -T-----------A-----------------A-----------T-------------------------------C---------TC------------------------------A--------------------------------
HPBCGADR   -------------A---------------T----G---------------------------------------GT------A--TC--C----------------C----G-AC-G--C///-C--------A----------------
HBVADRM    -------------A---------------T----G---------------------------------------GT------A--TC--C----------------C----G-AC-G--C///-C--------A----------------
HPBADRA    ----G------------------------T----G--------------------------T----------GT--C-----A---C--C----------------C---------T-A-----C-------------------------
HPBCG      ----G--------A---------------T----G--------------------------T----------GT--C-----A---C--C----------------C---------T-A-----C--------------G----------
HEHBVAYR   ----G--------A---------------T----G---------------------------------------GT------A--TC-A-----------------C---------T-A-----C-------------------------
HBVADR     ----G--------A---------------T----G--------------------------T------------GT------A--TC-------------------C---------T-A-----C-------------------------
HBVADR4    -------------A---------------T----G---------------------------------------GT------A--TC-------------------C---------T-A-----C-------------------------
HPBADR1C   ----G--------A---------------T----G--A------------------------------------GT------A--TC-------------------C---------T-A-----C-------------------------
HPBADRC    ----G--------A---------------T----G---------------------------------------GT------A--TC--C----------------C---------T-A-----C-------------------------
HBVPREX    ----G--------A-----C---------T----G---------------------------------------GT------A--TC-------------------C---------T-A-----C-------------------------
HPBETNC    ----G--------A--------------------G--------T---------T--------------------GT------A--TC--C----------------C---------T-A--C--C-----------------T-------
HHVBC      ----------C--------C-----T--------G--------------------------T------------GT------A--TC-------------------C---------T-------T-------------------------
HHVCCHA    -------------------------T--------G--A-----------T---T----------A---------GT------A--TC-------------------C---------T-------C-------------------------
HBVAYWMCG  -G--G--------------------T---T----G---------------------------------------GT------A---C-------------------C-----------A-------------------------------
HBVAYWC    -T--------C--------------T---T----G---------------------------------------GT------A---C-------------------C-----------A-------------------------------
HBVAYWC1   -T--------C--------T-----T---TT---G---------------------------------------GT------A---C-------------------CG----------A-----A-------------------------
HBVAYWE    -------------------C------------------------------------------------------------------------------------------------------------------------------
HBVDNA     -------------------C------------------------------------------------------------------------------------------------------------------------------
HPBHBVAA   -T--------C--------------T---T----G---------------------------------------GT------A---C-------------------C--------C--A-------------------------------
XXHEPAV    -T-----------------------T--GT-G--G--------T------------------------------GT------A---C-------------------C-----------A-------------------------------
HBVORFS    -T--------C--------C-----T---T--------------------------------------------GT------A---C--------------------A----------A-------------------------------
HHVBE4     -T---------C-----A-G-----T---T----G---------------------------------------GT------A---C-------------------C-----------A-----------------------T-------
HHVBBAS    -T---------C------------CT---T----G---------------------------------------GT------A---C-------------------C-----------A-----------------------T-------
HHVBF      -TC-C--------------C-----TACT--------------TGTC-------------------------GT------A-----------------------------------A--C-----------------------------
HHVBFFOU   -TC-C------C-----AAC-----CACT--G-----------TGTC--T----------------------CT-------------------------------------------A--C-----------------------------
HBVADW4A   -TC-C------C-------C-----TACT--------------TGTC-----------C-------------GT------A----------------G-----------------A--C-----------------------------
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            1201
HBVXCPS     CCACTGGCTGGGGCTTGGCCATAGGCCATCAGCGCATGCGTGGAACCTTTGTGGCTCCTCTGCCGATCCATACTGCGGAACTCCTAGCCGCTTGTTTTGCTCGCAGCCGGTCTGGAGCAAAGCTCATCGGAACTGACAATTCTGTCGTCC
HBVADW      ----------------A-------------------------------------------------------------------------------------------------------------------------------------
HVHEPB      -----------------------------------------C---------------------------------------------------------------------------G--A-----------------------------
S50225      ----------------A-------------------------------------------------------------------------------------------------------------------------------------
HPBADW2CG   -----------------------C-------------------A--------------------------------A-------------------------------------------A--------G--------------------
HPBADW3     --------------------------------------------G---------T--------------------T-----------A------------------A-------------A--T-----G--------------------
HPBADWZ     ------------------------------------------------------T---------------------------------------------------A-------------A--T-----G---------------T----
HPBADW1     --------T---------------------------------------------T---------------------------------------------------A-------------C--------G--C---------------A-
HPBADW2     --------T---------------------------------------------T-----------------------------------------------------------G-----A--------G------------------G-
HPBCGADR    --------A----------------------G-------------------------------------------------------A-----------------------------G--A--------G---------C--G--T--T-
HBVADRM     --------A----------------------G-------------------------------------------------------A-----------------------------G--A--------G---------C--G--T--T-
HPBADRA     --------A----------------------G-----------------------T-------------------------------A---------------GA---------------A--T-----G---------C--G--T----
HPBCG       --------A----------------------G-------------------------------------------------------A--------------------------------A--T-----G---------C-----T----
HEHBVAYR    -----G--T----------------------G-------------------------------------------------------A-----------------------------G--A--T---------C-----C--A--T----
HBVADR      --------A----------------------G-------------------------------------------------------A-----------------------------G--A--T---------C-----C-----T----
HBVADR4     --------A-----------T--C------AGC--------C---/-----------------------------------------A-----------------G-----------G--A--T-----C---C-----C-----T----
HPBADR1C    --------A-----------T--T-------GC---------------------------------------------------G--A--C-----------------------------A--T---------C-----C-----T----
HPBADRC     --------A--------------T-----ATC-G-----------------------------------------------------A-----------------------------G--A--T-----G---------C-----T----
HBVPREX     --------A--------------T---------------------------------------------------------------A-----------------------------G-CA--T---------C-----C-----T----
HPBETNC     --------A----------------------G-------------------------------------------------------A-----------------------------G--A--T-----G---------C-----T----
HHVBC       -----------------------G---------------------------------------------------------------G--------------------------------CA-T---------C-----C----------
HHVCCHA     -----------------------T---A-----------------------------------------------------------A--------------------------------A--T---------------C----------
HBVAYWMCG   -------------------T---G----------------------------CG----------------------------------------------------A-------------CA-TC----G---G--T--C-T---T----
HBVAYWC     -------------------T---G----------------------------TC----------------------------------------------------A-------------CA-T-----G------T--C-----T----
HBVAYWC1    -------------------GT--G----------------------------TC-------------------------------------C--------------A-------------CA-TC----G---G--T--C-----T--T-
HBVAYWE     --------------------A------A---------------------A----------------------------------------------------------------G------------------------------T----
HBVDNA      --------------------ATAG-----------------------------A----------------------------------------------------A-------------CA-T-----G------T--C-----T----
HPBHBVAA    -------------------T---G----------------------------CA------------------------------------------------------------------CA-TC----G------T--C-----T--T-
XXHEPAV     -------------------T---G----------------------------C------G----------------------------------------------A-------------CA-TC----G---G--T--C-----T--T-
HBVORFS     -------------------T---G----------------------------C-----------------------------------------------------A-------------CA-TC----G---G--T--C-----T--T-
HHVBE4      ---------------------------AT------------C----------------------------------------------------------------A-------------A--T-----G---C--T-----------T-
HHVBBAS     ---------------------------AT------------C----------------------------------------------------------------A-------------A--T-----G---C--T-----------T-
HHVBF       ------------------------------------------------------------------------------------T--A----C--C------------------------TC-------C---A----------------
HHVBFFOU    --------T--------------T------------------------------------------------------------T--A----C--C------------------------TC-------C---A----------------
HBVADW4A    --------T--------------T---------------------------------------------------------------A--------C---------A----------G-CT--------C---G-----------T----
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                                             Start of Hbx protein
           1351
HBVXCPS    TCTCGCGGAAATATACATCGTTTCC | ATGGCTGCTAGGCTGTACTGCCAACTGGATCCTTCGCGGGACGTCCTTTGTTTACGTCCCGTCGGCGCTGAATCCCGCGGACGACCCCTCTCGGGGCCGCTTGGGAGTCTCTCGTCCCCTTCTCC
HBVADW     -------------------A----- | ----------------------------------------------------------------------------------------------------------C------------------
HVHEPB     ----------------C-------- | ------A---------G-----------------------------------------------------------------------------------------C------------------
S50225     ------------------------- | ----------------G-----------------------------------------------------------------------------------------C---A--------------
HPBADWZCG  -T--T-A------------C----- | --A---------T-------------A---T--------------------C--------------------------------------G--A------------C-G-A--------------
HPBADW3    -T--C--C----------------- | ----------------G----------------G-----------------C--------------------------------------C--------------GC---AC--C--G-------
HPBADWZ    -T--C--C----------------- | ----------------G----------------G-----------------C-----T--------------------------------C--------------GC---AC--C--G-------
HPBADW1    ----C--C--G-------------- | ----------------G----------------G--------------------------------------------------------C--------------GC---AC--C--G-------
HPBADW2    ----C--C--G--------A----- | ----------------G----------------G-----------------------------------------------------T--C--------------GC---AC--C--G-------
HPBCGADR   ----T--------C--C--A--C-- | ---------C--G---G----------------G----------A------------------------------------------G-----C-----T-----CC--ATC-----------T-
HBVADRM    ----T--------C--C--A--C-- | ---------C--G---G----------------G----------A------------------------------------------G-----C-----T-----CC--ATC-----------T-
HPBADRA    ----T--------C--C--C--C-- | ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---AC------T-G--TT
HPBCG      ----T--------C--C--C--C-- | ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---AC-----------T-
HEHBVAYR   ----T--------C--C--C----- | ----------------G----------------G-----------------C-----------------------------------G-----------T-----CC---AC-----------T-
HBVADR     ----T--------C--C--C----- | ------------G---G----------------G-----------------C-----------------------------------G-----------T-----GC---AC-----------T-
HBVADR4    ----T--------C--C--C----- | ------------G---G----------------G-----------------C-----------------------------------G-----------T------C---AC-----------T-
HPBADR1C   ----T--------C--C--C----- | ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---A------------T-
HPBADRC    ----T--------C--C--C--C-- | ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---AC-----------T-
HBVPREX    ----T--------C--C--C----- | ------------G---G----------------G-----------------C-----------------------------------G-----------T-----CC---A------------T-
HPBETNC    ----T--------C--C--C--C-- | ---------C--A---G----------------G-----------------C-----------------------------------G--------T--T-----CC---AC-----------T-
HHVBC      ----T--------C-----C----- | ---------C--G---G----------------A-----------------------------------------------------G-----C-----T-----GC---AC-----------TT
HHVCCHA    ----T--C-----C-----C----- | ---------C------G----------------A--A--------------------------------------------------G-----------T-----GA---AC-----------T-
HBVAYWMCG  -A--C--C----------------- | ----------------G----------------GA-------------C--------------------------A-----------T------------------C------------------
HBVAYWC    ----C--C----------------- | ----------------G----------------G-----------------------------------------------------G--------T---------C------------------
HBVAYWCI   ----C--C----------------- | ----------------G----------------G-----------------------------------------------------T------------------C-----------------T
HBVAYWE    ------------------------- | -------------T-------------------------------------------------------------------------T------------------C------------------
HBVDNA     -A----------------------- | -------------T--------------------------------------------------------------------------------------------C------------------
HPBHBVAA   ----C--C--------G-------- | ----------------G----------------G-----------------------------------------------------T------------------C------------------
XXHEPAV    ----C--C-------------A--- | ----------------G----------------G-----------------------------------------------------T--------T---------C------------------
HBVORFS    ----C--C----------------- | ----------------G----------------G-----------------------------------------------------T-----------------GA---T--------------
HHVBE4     ----C--------------C----- | ----------------G----------------G--A--------------C-----------A-----------T-----------G--------T--------GA---T--------------
HHVBBAS    ----C-----G--------C----- | ----------------G----------------G--A--------------C-----------A-----------T-----------G--------T--------GA---A--------------
HHVBF      ----T-----G--------C----- | ---------C--T---G----------------G-----------------------------------------A--------A-----C-----T--------GC-G-AC--C--------T-
HHVBFFOU   ----C-----G--------C----- | ---------C--A---G----------------G-----------------------------------------A--------A-----C--------------GC---AC--C--T-----G-
HBVADW4A   ----TA----G--C--C--C----- | ---------C--A---G---A------------G--------------------------A-----------------------------C--------------GC-G-AC--C--T-------
```

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                                                                                                                         End of HB pol protein
          1501
HBVXCPS   GTCTGCCGTTCCAGCCGACCACGGGGCGCACCTCTCTTTACGCGGTCTCCCCGTCTGTGCCTTCTCATCTGCCGGTCCGTGTGCACTTCGCTTCACCTCTGCACGTTGCATGGAGACCACCGTGA | ACGCCCATCAGATCCTGCCCAAGGT
HBVADW    -----------------------------------------------------------------------------------------------------------------C----------- | -------------------------
HHVHEPB   ----------------------------------------------------------------------------------------------------------------------------- | -------------------------
S50225    ----------------------------------------------------------------------------------------------------------------------------- | ------------G------------
HPBADW2CG ---------A--GT--------------------------------------------------------------------------------------------------------------- | -----------G-------------
HPBADW3   -C-------A--GT-------------------------------A-----------------------------A------------------------------C------------------ | ---------G--A------------
HPBADW2   ---------A--GA-------------------------------A--------------------G--------A------------------------------C------------------ | ---------G--A------------
HPBADW1   ---------A--GT-------------------------------A-----------------------------A------------------------------C------------------ | -------C-G--A------------
HPBADW2   -C---TT--A--GA-------------------------------A-----------------------------A------------------------------C------------------ | -------CGG--A------------
HPBCGADR  A-----G-----G--------------------------------------------------------------A------------------------------C------------------ | -----G-----G--T----------
HBVADRM   A-----G-----G--------------------------------------------------------------A------------------------------C------------------ | -----G-----G--T----------
HPBADRA   C--------------------------------------------------------------------------A------------------------------C------------------ | ---G---C---G--T--------C-
HPBCG     A---------------------------------------------------------------------------------------------------------C------------------ | -------C---G--T----T---C-
HEHBVAYR  A-----------G--------------------------------------------------------------A------------------------------A------------------ | -------C---G--T----------
HBVADR    T-----------G--------------------------------------------------------------A------------------------------C------------------ | -------C---G--T----------
HBVADR4   A-----------G-----------------------------------TTTT------------------------------------------------------C------------------ | -------C---G--T----------
HPBADR1C  A---A-------G--------------------------------------------------------------A-------------C----------------C------------------ | -------C---G--T----------
HPBADRC   A-----------G----------------G----------------------------------------------------------------------------C------------------ | -------C---G--T----------
HBVPREX   -----T------G--------------------------------------------------------------A------------------------------C------------------ | -------C---G--T----------
HPBETNC   A-----------G--------------------------------------------------------------A------------------------------C------------------ | -----------G-GT----------
HHVBC     ------G-----G---A----------------------------------------------------------A------------------------------C-------A---------- | -------CAT-G--T----------
HHVCCHA   ------G-----G---A----------------------------------------------------------A------------------------------C------------------ | -------CAT-G-AT----------
HBVAYWMCG ----------T-GT-------------------------------A-----------------------------A------------------------------C------------------ | -------C--AT--T----------
HBVAYWC   ------------GA-------------------------------A---------------------------T-A-T----------------------------C------------------ | -------C--A--AT----------
HBVAYWC1  ----------T-GA-------------------------------A-----------------------------A------------------------------C------------------ | -------------------------
HBVAYWE   --------------------------------------------------------------------------------------------------------A-------------------- | -------------TA----------
HBVDNA    -------------------------------------------------A--------------------------------------------------------C------------------ | ----------A-GT-----------
HPBHBVAA  ----------T-GA-------------------------------A-----------------------------A------------------------------C------------------ | -------C--AT--T----------
XXHEPAV   ----------T-GA-------------------------------A-----------------------------A------------------------------C------------------ | -A-----A-CAT--T----------
HBVORFS   ------------GT-------------------------------A------------------------------------------------------------C------------------ | -------C--CT--T----------
HHVBE4    ------G-----G--------------------------------------------------------------A------------------------------C------------------ | -------C--A---T----------
HHVBBAS   ----------------------------------------------------------T----------------A------------------------------C------------------ | -------C--A--AT----------
HHVBF     -------------------A-----T-------------------A------------T----------------A------------------------------C------------------ | ------CCTG--GTT----A-CA--
HHVBFFOU  -------------------------T-------------------A------------T-----------------------------------------------C------------------ | ------CCTG--GTT----A-CA--
HBVADW4A  -------------------G-----T-------------------A------------T----------------A------------------------------C------------------ | ------CCTG--GTT----A-CA--
```

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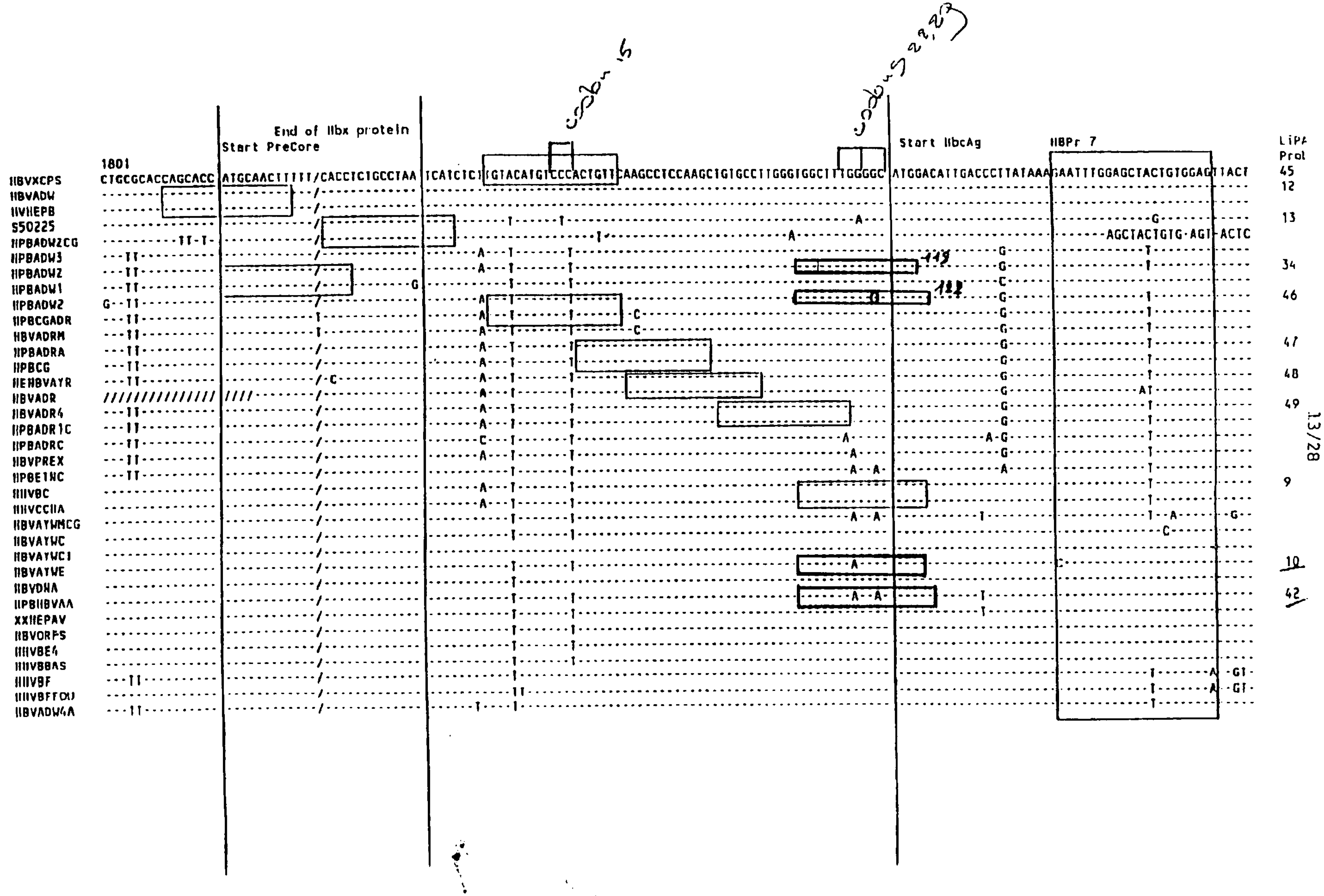
                   HBPr 69                                  HBPr 70                       preCore       Core
                                                                                          promoter      promoter        LiPA
            1650                                                                                                        Probe
HBVXCPS     CTTCATAAGAGGACTCTTGGACTCTCAGCAATGTCAACGACCGACCTTGAGGCCTACTTCAAAGACTGTGTGTTTAAGGACTGGGAGGAGCTGGGGGAGGAGATTAGGTTAAAGGTCTTTGTATTAGGAGGCTGTAGGCATAAATTGGT
HBVADW      ------------------------C----------------------------------------------------------------T---------------------T-A-----------------------------------
HVHEPB      ------------------------C----------------------------------------------------------------------------------------------------------------------------
S50225      -----------A------------C--------------------------------------------------------------------------------------T-A-----------------------------------
HPBADWZCG   -----------------------------------------------------------------------------A-----------T-----------------------------------------------------------
HPBADW3     ---G-------------------T-----------------------------A-----------------------T--G--------T-------------C------------------C--------------------------
HPBADWZ     ---G-------------------T------------C----------------A-----------------------T--G--------T-------------C------------------C--------------------------
HPBADW1     ---G-------------------T-----------------------------A-----------------------T--G-----------------------------------------C-C------------------------
HPBADW2     ---G-------------------T-----------------------------A-----------------------T--G--------T------------G-------------------C--------------------------
HPBCGADR    ---------------------------------------T-------------A--------------CT-------A----------CT---------------------T-A--------C--------------------------  89
HBVADRM     ---------------------------------------T-------------A--------------CT-------A----------CT---------------------T-A--------C--------------------------
HPBADRA     --------------------------------------A--------------A---------------T-------A-----------T--------------------------------C--------------------------
HPBCG       -----------------------------------------------------A---------------T-------A-----------T--------------------------------C--------------------------  88
HEHBVAYR    ------C----------------------------------------------A---------------T-------A-----------T--------------------------------C--------------------------
HBVADR      ------------------------------C----------------------A-----------------------A-----------T--------------------------------C-----------------/////////
HBVADR4     ------------------------------G----------------------A---------------T-------------------T--------------------------------C--------------------------
HPBADR1C    -----------C-----------------------------------------A---------------T-------------------T--------------------------------C-G------------------------
HPBADRC     ------------------------------G----------------------A--T------------T-------A-----------T---------------------T----------C--------------------------
HBVPREX     ---T-------------------------------------------------A---------------T-------A-----------T---------------------T-A--------C--------------------------
HPBETNC     ---T-------------------T-----------------------------A---------------T-------A-----------T-----------AC--------T-A--------C--------------------------
HHVBC       ---G-------A-----------------------------------------A--T-----------------C--A-----------T------------G---A---------------C--------------------------
HHVCCHA     ---G--------------------------G----------------------A-----------------A-----A-----------T----------------A---------------C--------------------------
HBVAYWMCG   -----------------------------------------------------A---------------T-------A--G--------T----------------A------T-G---------------------------------
HBVAYWC     --------------------------T--------------------------A---------------T-------A-----------T----------------A---------------C--------------------------
HBVAYWC1    ------------------------C----------------------------------------------------------------------------------------------------------------------------
HBVAYWE     T-----------------------C----------------------------------------------------------------------------------------------------------------------------
HBVDNA      ------------------------C----------------------------------------------------------------------------------------------------------------------------
HPBHBVAA    --------------------------T-T------------------------A---------------T------------------TC----------------A----T-A--------C--------------------------
XXHEPAV     --------------------------T-T------------------------A---------------T-------A-----------T----------------A------------------------------------------
HBVORFS     -----------------------------------------------------A---------------T-------------------T----------------A---------------C--------------------------
HHVBE4      --------------------------T--------------------------A---------------T-------A-----------T----------------A---------------C--------------------------
HHVBBAS     --------------------------T--------------------------A---------------T-------A----------TC----------------A---------------C--------------------------
HHVBF       -----------------------T----G-CG------T---TGGA-C--A-A----A-------------A-------------------------------C------------------C--------------------------
HHVBFFOU    ----------------A------T----G-CG------T---TGGA-C--A-AA---A-------------A-----A-------------------------C------------------C--------------------------
HBVADW4A    ----------C------------T----G-TG------T---TGGA-C--A-AA---A-------------A-----------------T-----------------------------------------------------------
```

12/28


End of Hbx protein
Start PreCore
codon 15
codons 22, 23
Start HbcAg
HBPr 7
LiPA Prol
1801
CTGCGCACCAGCACC ATGCAACTTTTT/CACCTCTGCCTAA TCATCTC TGTACATGTCCCACTGTTCAAGCCTCCAAGCTGTGCCTTGGGTGGCTTTGGGGC ATGGACATTGACCCTTATAAAGAATTTGGAGCTACTGTGGAGTTACT
HBVXCPS
HBVADW
HVHEPB
S50225
HPBADWZCG
HPBADW3
HPBADWZ
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWC1
HBVAYWE
HBVDNA
HPBHBVAA
XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A
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12
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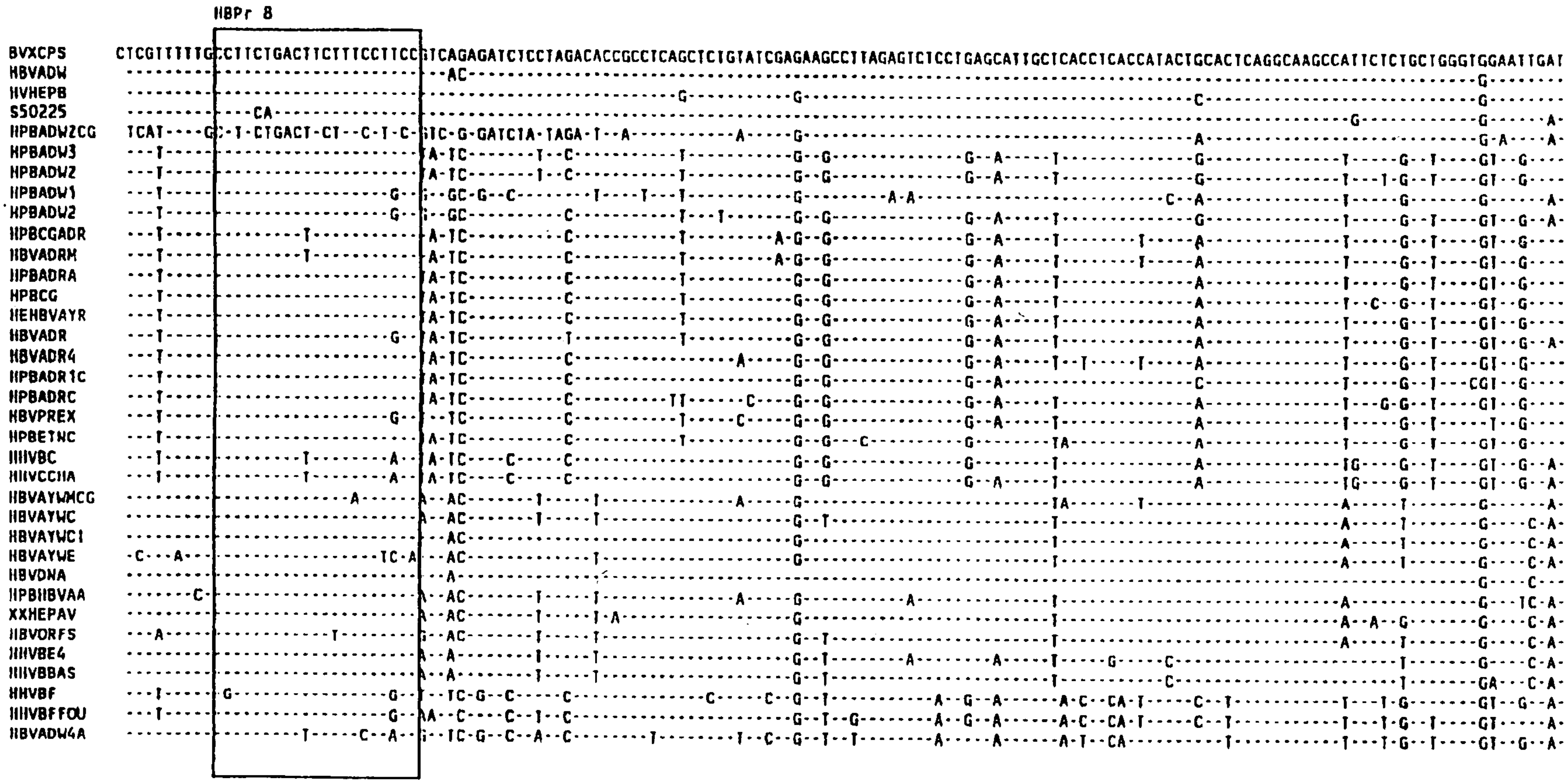
42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HBVADW
HVHEPB
S50225
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HPBADW3
HPBADW2
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWC1
HBVAYWE
HBVDNA
HPBHBVAA
XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A
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          2101
HBVXCPS   GACTCTAGCTACCTGGGTGGGTAATAATTTGGAAGATCCAGCATCCAGGGATCTAGTAGTCAATTATGTTAATACTAACATGGGTTTAAAGATCAGGCAACTATTGTGGTTTCATATATCTTGCCTTACTTTTGGAAGAGAGACTGTGCT
HBVADW    -------------------------------C----------------A--------------------------------------------------------------------------------------------------A--
HVHEPB    ------------------------------------------------------------------------------------A-----------------C----------G---C----A------------------A-----A--
S50225    -------------------------------C---------------------A---------------------------------------------------------------------T----TG-----------C-----A--
HPBADW2CG ------------------------------------------------------------------------------------CC--------------T----------------T-----------------------A-----C--
HPBADW3   --A------C-----------A-G------------C-----C---C----AT---------G------C---GT---T-----CC----A-----A-----------------C--T--C--T-----G-----------A-----T--
HPBADWZ   --A------C-----------A-G------------C---------C----AT---------G------C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A-----T--
HPBADW1   ----------------------------------------------C-------------------------C-----------CC------------------G---------C--T--C--T-----------------A-----T--
HPBADW2   --A------C-----------A-G-----------------G-----------AT---------GC-----C--CGT---T-----CC----A-----A-----------------C--T--C--T-----------G-----A-----T--
HPBCGADR  --A---G--C-----------A-G------------C--------------A----------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--------------------A-----TT-
HBVADRM   --A---G--C-----------A-G------------C--------------A----------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--------------------A-----TT-
HPBADRA   --A-T-G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A--------------------T--C--T-----------------A-----T--
HPBCG     --A-T-G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A-----T--
HEHBVAYR  --A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A--T--A-----------------C--T--C--------------------A-----C--
HBVADR    --A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--------------------A-----TT-
HBVADR4   --A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----C-----------C--T--C--T-----------------A-----T--
HPBADR1C  --A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T--C--CC----A-----A-----C-----------C--T--C--T-----------------A-----T--
HPBADRC   --A---G--C-----------A-G------------C--------------AT-G-------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A--G--T--
HBVPREX   --A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A-----TT-
HPBETNC   --AC--G--C-----------A-G----------------A----------AGC--------GC-----C---GT---T-----CC----AC----A-----------------C--T--C--T-----------------T-----T--
HHVBC     --A---G--------------A-G------------C--------A--A--AT-G-------G------C---GT---T-----CC----A-----------G----------------T--C--T-----------------A-----T--
HHVCCHA   --A---G--------------A-G------------C--------------AT----G----G------C--C-T---T-----CC----A-----A-----------------C--T--C--T-----------------A-----T--
HBVAYWMCG ---------C------------GG-------C--------A----------C----------G---------C-----T-----CC-----T----------------------CGT-----T--C--------------A--A--CG-
HBVAYWC   ----------------------GT---------------------T-----C----------G------C--C-----T-----CC-----T----A-----C-----------C--T-----T--C-T-------------A--A--TA-
HBVAYWCI  ----------------------GT---------------------T--A--C----------G------C--C-----T-----C------T----------C-----------C--T-----T--C---------------A--A--TA-
HBVAYWE   ----------------------GT---------------------T--A--CT---------G------C--C-----T-----CC-----T----------C-----------C--T-----T--C---------------A--A--TA-
HBVDNA    ---------AT-----------G-------------------G--T-----C----------G---------C-----T-----CC-------------------------------------------------------------A--
HPBHBVAA  A------T-C------------GG----------------A----T-----C----------G---------C-----T-----CC-----T----------------------C--T-----T--C---------------A--G--CA-
XXHEPAV   ----------------------GG----------------AT---------C----------G------C--C-----T-----CC----AT-----------------------C--T-----T--C---------------A--A--TA-
HBVORFS   ----------------------GT---------------------T-----C----------G------C--C-----T-----CC-----T----------------------C--T-----T--C---------------A--A--CA-
HHVBE4    ----------------------GTA--------------------------C----------G------C--------T-----CC-----T--------T-------------C--T-----T--C---------------A--C--CA-
HHVBBAS   ----------------------GTA--------------------------C----------G------C--------T-----CC-----T--------T-------------C--T-----T--C---------------A--C--CA-
HHVBF     ----T-G---T-------------------------C--T---G-T------T-------T--------C--C-----T-----C--------T--A-----------------C--C--C--T-----------------A--A--T--
HHVBFFOU  ----T-G---T----------C-----------G--C--T---G--------T-------T--C--------C-----T-----C--------T--A-----------------C--T--C--------------------A--A--T--
HBVADW4A  ----T-G---T----------C-----------G--C--T---G-T------T-------T--C--------C-----------CC----A--T--A-----G-----------C--T--C--------------------A--A--T--
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15/28

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                                                                        Start HB pol protein
            2251
HBVXCPS     TGAATATTTGGTCTCTTTCGGAGTGTGGATTCGCACTCCTCCAGCCTATAGACCACCAA ATGCCCCTATCTTATCAACACTTCCGGAAACTACTGTTGTTAGACGACGGGACCGAGGCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCG
HBVADW      ----------------------------------------------------------- -------------------------------------------------------------------------------------------
HVHEPB      ----------------------------------------------------------- -------------------------------------------------------------------------------------------
S50225      ----------------------------------------------------------- --------------------------------------------T-T---------C----------------------------------
HPBADWZCG   ---G------------------------------------------------------- -------------------------------------------------A-----------------------------------------
HPBADW3     ------------G-----T-----------------A--------A------------- -------------------------------------------------//////------------------------------------
HPBADWZ     ------------G-----T-----------------A-----T--A------------- -------------------------------------------------//////-------------------------------A----
HPBADW1     G-----------A-----T-----------------------T-----C---------- -------------------------------------------------//////------------------------------------
HPBADW2     ------------G-----T-----------------------T--A------------- -----------------------------------------------A-//////------------------------------------
HPBCGADR    G--G--------A-----T-----------------------C--T--C---------- -------------------------------------------------//////------------------------------------
HBVADRM     G--G--------A-----T-----------------------C--T--C---------- -------------------------------------------------//////------------------------------------
HPBADRA     ---G--------G-----T-----------------------C--T--C---------- -------------------------------------------------//////------------------------------------
HPBCG       ---G--------G-----T-----------------------C--T--C---------- -------------------------------------------------//////------------------------------------
HEHBVAYR    ---G--------G-----T-----------------------C--T--C---------- -------------------------------------------------//////------------------------------------
HBVADR      G--G--------A-----T-----------------------C--T--C---------- -------------------------------------------------//////------------------------------------
HBVADR4     ---G--------G-----T-----------------------T--T--C---------- -------------------------------------------------//////------------------------------------
HPBADR1C    ---G--------G-----T-----------------------T--T--C---------- -----------------------------------------------//////--------------------------------------
HPBADRC     ---G--------A---G-T----------------------A------C---------- -------------------------------------------------//////------------------------------------
HBVPREX     A--G--------G-----T----------------------TC--T--C---------- ---------------------------------------------A---//////------------------------------------
HPBEINC     ---G--------G-----T---------------------A-C--T--C---------- -------------------------------------------------//////------------------------------------
HHVBC       ---G--C-----G--C--T-----------------------C--T--C---------- -----------------------------------------------A-//////------------------------------------
HHVCCHA     ---G--------G-----T-----------------------C--T--C---------- ----------------------------------------------T--//////------------------------------------
HBVAYWHCG   A--G--------G-----T----------------------A---T------------- ----------------------------G---TG---------------//////------------------------------------
HBVAYWC     A--G--------G--------------------------------T------------- -----------C----------------G--------------------//////------------------------------------
HBVAYWC1    A--G--------GG-------------------------------T------------- -----------C----------------G--------------------//////------------------------------------
HBVAYWE     A--G--------G--------------------------------T------------- -----------C----------------G-------------C------//////----------------------------------GC
HBVDNA      ----------------------------------------------------------- ----------------------------------------------------//////---------------------------------
HPBHBVAA    A--G--------G-----T--------------------------T------------- ----------------------------G--------------------//////------------------------------------
XXHEPAV     A--G--------G-----T--------------------------T------------- ----------------------------G--------------------//////------------------------------------
HBVORFS     A--G--------G--------------------------------T------------- -------------------------------------------------//////------------------------------------
HHVBE4      A--G--------G-----T--------------------------T------------- ----------------------------G-A----------------A-//////------------------------------------
HHVBBAS     A--G--------G-----T--------------------------T------------- ----------------------------G-A----------------A-//////------------------------------------
HHVBF       ---G--------G--C--T--------------------A--T--T------------- -----------C---C---------------------------------//////------------------------------------
HHVBFFOU    ---G--------G--C--T--------------------------T------------- -----------C---C---------------------------------//////------------------------------------
HBVADW4A    A--G--------G--C--T-----------------------T--T--C---------- -----------C---C---------------------------------//////------------------------------------
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                                                                   End of HBcAg
          2401
HBVXCPS   CAGACGCAGATCTCAATCGCCGCGTCGCAGAAGATCTCAATCTCGGGAATCTCAATGTTAG|TATTCCTTGGACTCATAAGGTGGGAAACTTTACTGGGCTTTATTCCTCTACAGTACCTATCTTTAATCCTGAATGGCAAACTCCTTCCT
HBVADW    -------------------------------------------------------------|---------------------C-----------G-------------------------------------------------------
HVHEPB    -------------------------------------------------------------|------------------------------C----------------------C-----------------------------------
S50225    -------------------------------------------------------------|-----------------------------------------------------------------------------------------
HPBADWZCG -------------------------------------------------------------|---------------------------T-----------------T-----T--C--------------------------A-----T-
HPBADW3   ------A--G---------------------------------------------------|---------------------------------G-----------T-----G-------G----------C----------------A-
HPBADWZ   ------A--G------------CG-------------------------------------|---C-----------------------------G-----------T-----G-------G----------A------------------
HPBADW1   ------A--G--------A--------------------------------C---------|---C-----------------------------G-----------T------------G----------------------------T-
HPBADWZ   ------A--G---------------------------------------------------|------------A--------------------G-----------T-----G------TG----------A----------------T-
HPBCGADR  ------A------G-----------------------------------------------|---C-----------------------------------------T-----T------G-------------G-----------C----
HBVADRM   ------A------G-----------------------------------------------|---C-----------------------------------------T-----T------G-------------G-----------C----
HPBADRA   ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G--C----------G-CC--------C----
HPBCG     ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G-------------G--C--------C----
HEHBVAYR  ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G-------------T---A-------C----
HBVADR    ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G----------C--G-----------C----
HBVADR4   ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G-------------G-----------C----
HPBADR1C  ------A--G---------------------------------------------------|---C-----------------------------------------T-----T--------------------G-----------C----
HPBADRC   ------A--G---A-----------------------------------------------|---C--------------------------------T--C-----T-----T------G-------------G-----------C----
HBVPREX   ------A------------------------------A-----------------------|---C--------------------------------A--------T-----T------G-------------G---A-------C--T-
HPBETNC   ------A--G---------------------------------------C----------A|-G-C-----------------------------A--A-----C--T-----T------G-------------G-----------C----
HHVBC     ------A--G-----------------------------------------C---------|---C-----------------------------------------T-----T------G-------------------------C--T-
HHVCCHA   ------A--G--------A--------------------------------C---------|---C-----------------------------------------T-----T------G-------------------------C--T-
HBVAYWMCG ------A--G---------------------------------------------------|---------------------------------G-----------T-----T--T---G-------C---C-T---A----A--C--T-
HBVAYWC   ------A--G---------------------------------------------------|------------------------G--T-----------------T-----T------G-----------C-T---A----A--A--T-
HBVAYWCI  ------A--G---------------------------------------------------|---------------------------------G---T-------T-----T--T---G-------C---C-T---G----C--C--T-
HBVAYWE   -----CA--G---------------------------------------------------|------------------------G--------G-----------T-----T--T---G-----------C-T---A----A-----T-
HBVDNA    ------A--G---------------------------------------------------|------------------------G--------------------T-----T------G-----------C-T---A----A--A--T-
HPBHBVAA  ------A--G-----------A------------A--------------------------|------C--------------------------G-----------T-----T--T---G-------C---C-T---A----A--C--T-
XXHEPAV   ------A--G---------------------------------------------------|---------------------------------G-----------T-----T------G-------C---C-T---A----A--C--T-
HBVORFS   ------A------------------------------------------------------|-------------------A----T--------G-----------------T------G-------C---C-T---A----A--C--T-
HHVBE4    ------A-------------------------------------CA-CT--C---------|---------------------------T-----G-----C--C--T-----TA-T---G-----------A-C---A-------A--T-
HHVBBAS   ------A-------------------------------------CA-CT--C---------|---------------------------T-----G--------C--T-----TA-----G-----------A-C---A-------A--T-
HHVBF     -C----A--G----------------------------------CA-CT--C---------|---------------------------T-----G-----C--C--T-----T------GCT--C------A-C---TT---------T-
HHVBFFOU  ------A--G----------------------------------CA-CT--C---------|---------------------------T-----G-----C--C--T-----T------GCT--C------C-C---TT---------T-
HBVADW4A  ------A---------------------C-C-------------CA-CT--C---------|---------------------------------G--------C--T-----T--G---GCT---------A-C---TCC--------T-
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           2551
HBVXCPS    TTCCTAAGATTCATTTACAAGAGGACATTATTAATAGGTGTCAACAATTTGTGGGCCCTCTCACTGTAAATGAAAAGAGAAGATTGAAATTAATTATGCCTGCCAGATTCTATCCTACCCACACTAAATATTTGCCCTTAGACAAAGGAA
HBVADW     -------------------------------------------------------------------------------------------------------T----------------------------------------------
HVHEPB     -------A-----------------------------------------------------------------------------------------------T----------------------------------------------
S50225     -------------------------------------------------------------T-----------------------------------------T--------------------A-------------------------
HPBADW2CG  -------A-----------T--A------GC------------G--------A-----------------------A------C-------------------T--G--T-------A-AG---A-----------------------G-
HPBADW3    -----G-C--------G--G--------C-------A----A-------------A--C--T--A--G--------A--G---C-A-----G-----------T--G--------C-ATGTT--------------------T-------
HPBADWZ    -----G-C--------G--G-----T--C------------G-------------A--C-----A--G--------C--G---C-A-----G-----------T--G--------C-ATGTT--------------------T-------
HPBADW1    ----AG-C-----------G---------G--G----A---A-G-----------A--C--T--A-----------C--G---C-A--------A--------T-----T-----C-ATGTT--C-----------------T-----T-
HPBADW2    -----G-C--------G--G---------G--G----A---A-G-----------G--C--T--A-----------C--G---C-A--------------C--T--G--T-----C-ATGTT--------------------T-----G-
HPBCGADR   -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G--T-------AA-TT--C--------------G--T-----C-
HBVADRM    -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G--T-------AA-TT--C--------------G--T-----C-
HPBADRA    -------C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--T-----C-
HPBCG      -------C-----------------T-----------A----------A------------T--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--T-----C-
HEHBVAYR   -----C-C-----------G-----------------A----------A------------G--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--------C-
HBVADR     -----C-C-----------G-----------------A----------A------------T--G--T--------A--G-----A-----------------T--G----------A--TT--------------------------C-
HBVADR4    -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G----------A--TT--C-----------A--G--------C-
HPBADR1C   -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A--G--------------T--G----------A--TT--C------C-------G--------C-
HPBADRC    -------T-----------G-----T-----------A----------A---A--------T--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--T-----T-
HBVPREX    -------C-----------G------------G----A----------A------------T--A--G--------A--G-----A-----------------T--G----------A--TT--C--G-----A-----G--T-----C-
HPBETNC    -------C-----------G------------G----A----------A------------T--A--T--------A--G-----A-----------------T--G--T-----A-A--TT--C--------------G--T-----C-
HHVBC      -----G-C--------G--G-----------------A----------A------------T--A--T--------A--------A-----------------T--G--T-------A--TT--------------------------C-
HHVCCHA    -----G-C--------G--G-----------------A----------A------------T--A--T--------A--------A-----------------T--G--T-------A--TT--C-----------------T-----C-
HBVAYWMCG  -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G----------T---G--T-----A-TGGTT--C-----------A--G--T--G--T-
HBVAYWC    -------T--A--------CC-A--------C--A-AA---G----G-----A-----A-----A--T-----G--A---------C----G--C--------T--G--T-----A-AGGTT--C--------A--A--G--T--G--T-
HBVAYWC1   -------T--A--------CC-A--------C--A-AA---G----G-----A-----A-----A--T-----G--A---------C----G-----------T--G--T-----A-AGGTT--C--------A--A--G--T--G--T-
HBVAYWE    -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G-----------T--G--T-----A-ATGT---C-----------A--G--T--G--T-
HBVDNA     -------T--A--------CC-A--------C--A-AA---G----G-----A-----A-----A--T-----G--A---------C----G-----------T--G--T-----A-AGGTT--C--------A--A--G--T--G--T-
HPBHBVAA   -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G-----------T--G--T-----A-AGGTT--C-----------A--G--T--G--T-
XXHEPAV    -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G--------A--T--G--T-----A-ATGTT--C-----------A--G--T--G--T-
HBVORFS    -------T--A---C----CC-A--------C--A-AA---G----------A-----A-----A--------G--AC-----C--C----------------T--G--T-----A-ATGTT--------------A-----T--G--T-
HHVBE4     -----G-T--------G--CC-------------C-AA---G----------A--T-----A--A-----------AC-------A--C---G-C--------T-----T-T---C-T-TCT--A------------C----G-----T-
HHVBBAS    -----G-T--------G--CC-------------C-AA---G----------A--T-----A--------------AC-------A--C---G-C--------T-----T-T---C-T-TCT--G------------C----G-----T-
HHVBF      -----G-T-----------TC----T--G--ATC--AA---G----------A-----G------AA------TT----------A-----GG-C-----A--T-----T-------AG--T--C--------CTA--G---G-----G-
HHVBFFOU   -----G-T--------G--TC-A---C-G--ATC--AA---G----------A-----A--T--CAA------TT-------G--------G--------A----------T-----AA-TT-----------C--TC-G--G----AC-
HBVADW4A   -----G-T--------G--TC-A---C-G---TC--AA---G----------A-----A--T---AA------TTAC--------A-----GG-------A--T-----T-------AGGTT--C--------T---A-G--T-----C-
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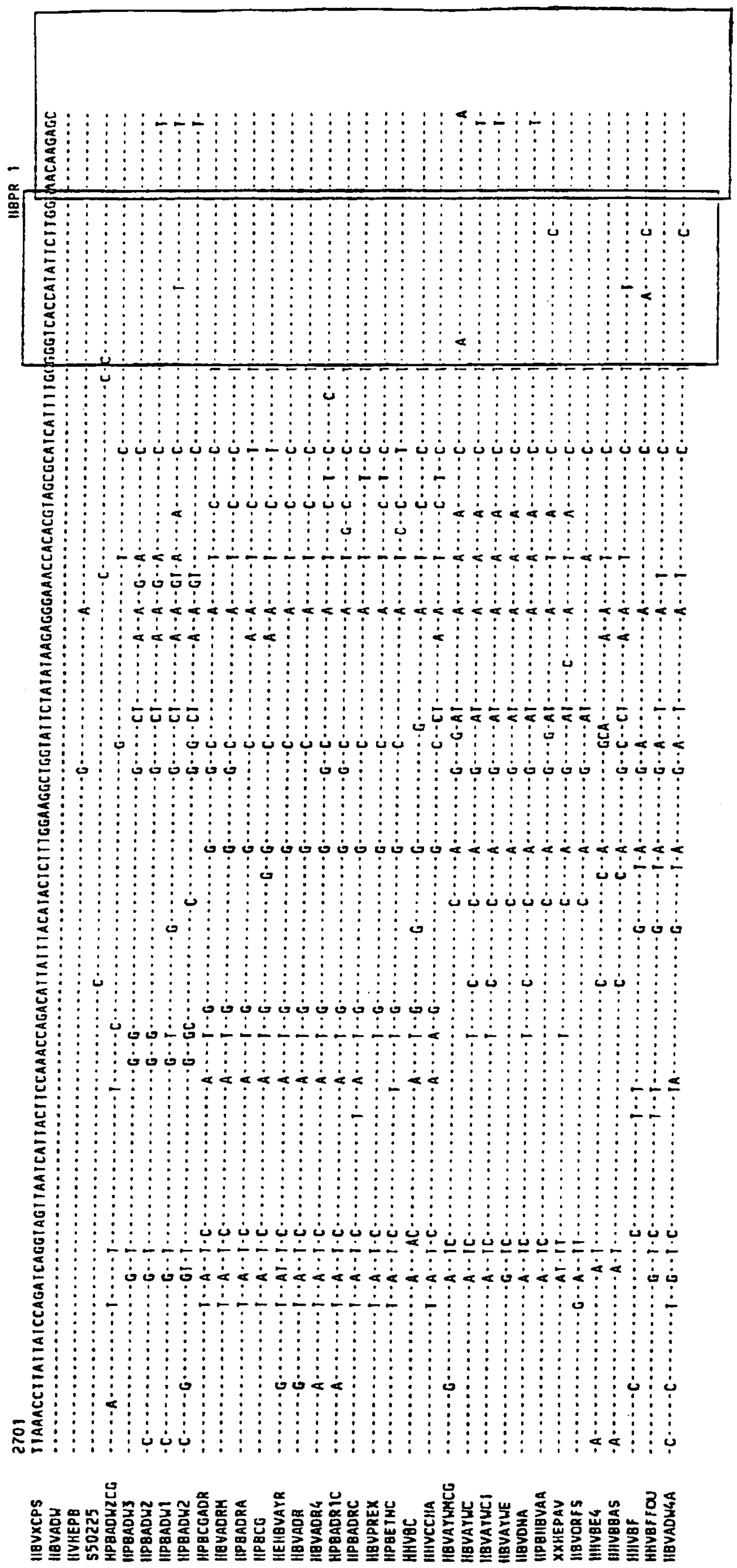
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HBVXCPS
HBVADW
HVHEPB
S50225
HPBADWZCG
HPBADW3
HPBADW2
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWC1
HBVAYWE
HBVDNA
HPBHBVAA
XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A

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                     Start HBpreS1Ag
                     HBPr 2                                                                                                                                    LIP.
                                                                                                                                                               Pro
HBVXCPS     TACAGC ATGGGAGGTTGGTCATCAAAACCTCGCAAAGGCATGGGGACGAATCTTTCTGTTCCCAACCCTCTGGGATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCATC 50
HBVADW      ------ --////////////////////------------------------------------C-----------------------------------------------CA--------------------
HVHEPB      ------ -------------------------------------------------------------------------------T---------------------------------------------- 22
S50225      ------ ------G--A------------------------------------------------------------------------------------------------------------------
HPBADWZCG   ----T- ------------------------------C-------------------------------------------------------T------------------------------------
HPBADW3     ------ -----------C--C--------AC-----------A--------C--C-----T-----------------------C------------AA------CG------C---------C-------ACA- 57
HPBADWZ     ------ -----------C--C--------AC-----------A--------C--C-----T-----------------------C------------AA------CG------C---------C-------ACA-
HPBADW1     ------ -----------T--C--------A------------A-----------C-----T--C-----------C--------------------AA-------G-A-------------C-------ACA-
HPBADW2     ------ -----------T--C--------A------------A-----------C-----T--------------C--------------------AA-------G-A-------------C-------G-A-
HPBCGADR    ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HBVADRM     ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HPBADRA     ---G-- -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A- 55
HPBCG       ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
XEHBVAYR    ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HBVADR      ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HBVADR4     ------ -----------T--C--------AC---------------------------T-----------------------C-----------G-----------C------C---------------A-
HPBADR1C    ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HPBADRC     ------ -----------T--C--------AC---------------------------T-----------------------C----------------------------------------------A-
HBVPREX     ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HPBETNC     ------ -----------T--C--------AC---------------------------T-----------------------C-----------G----------------------------------A-
HHVBC       ------ -----------T-----------G------------------G--A------T-----------------------C-----------G---------------------C------------A-
HHVCCHA     ------ -----------T--C--------G----------------------------T-----------------------C----------------------------------------------A-
HBVAYWMCG   ------ -----/////////////////////////////////////-CA-------CACCAG---T-------------------C--C---------T--A---C---A----A---A-CGCA-------------T----A- 92
HBVAYWC     ------ -----/////////////////////////////////////-CA-------CACCAG---T-------------------C--C---------T--A---C---A----A---A-CGCA-------------T----A-
HBVAYWC1    ------ -----/////////////////////////////////////-CA-----A--CACCAG---T-------------------C--C---------T--A---C-C-A----A---A-CGCA-------------T----A-
HBVAYWE     ------ -----/////////////////////////////////////-CA--------CACCAG---T-------------------C--C---------T--A---C---A----A---A-CGCA-------------T----A-
HBVDNA      ------ -----/////////////////////////////////////-CA-----A--CACCAG---T-------------------C--C---------T--A---C---A----A---A-CGCA-------------T----A-
HPBHBVAA    ------ -----/////////////////////////////////////-CA--------CACCAG---T-------------------C--C---------T--A---C---A----A---A-TGCA-------------T----A- 73
XXHEPAV     ------ -----/////////////////////////////////////-CA--------CACCAG---T-------------------C--C---------T--A---C---A----A---A-CGCA-------------T----A-
HBVORFS     ------ -----/////////////////////////////////////-CA--------CACCAG---T-------------------C--C---------T--A---C---A----A---A-CGCA-------------T----A-
HHVBE4      ----T- -----GCT--CT-GGA-GGTC---T-G--///TG------A----A----CACCA----T-------------T-------C--C---------T--A-------A----A---A-C-GA------------CA---T----A- 60
HHVBBAS     ----T- -----GCT--CT-GGA-GGTC---T-G--///TG------A----A----CACCA----T-------------T-------C--C---------T--A-------A----A---A-C-GA------------CA---T----A- 61
HHVBF       ------ ------CACCTCTC----CGA-AA-A-GG---------ACA-----------G------T--A-----C-----G--A--C-------C----T--GCT----A---A--T--C-G--G---C--C--------------A-A-A- 64
HHVBFFOU    ------ ------CACCTCTC----CGA-AA-A-GG---------ACA------C-----G------T--A--------------A--C-----AC----T---CTT---A---A--T--C-G--G---C----------------AAA-A-
HBVADW4A    ------ ------CACCTCTC----CGA-A--A-G----------ACA------C-----G------T--------------------A--C-------C----T--GCT----A---A--T--C-G--G---C--C--------------A-A-A- 63
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            3001                                                                                                                                                           LiPA
HBVXCPS     AAGGACCACTGGCCAGCAGCCAACCAGGTAGGAGTGGGAGCATTCGGGCCAAGGCTCACCCCTCCACACGGCGGTATTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACCACAGTGTCAACAATTCCTCCTCCTGCCTCCACCAAT   Prob
HBVADW      ----------------------------------------------G-------------------------------------------------------------------------------------------------------   15
HVHEPB      ----------------------------------------------G--T-------------------G-------------------------G-----C---------------------------G----   33
S50225      ---------------------G------------------------G---------------------------------------------A---------------------------------------------
HPBADW2CG   -------------CA-----------------T-----G--T----T--C-------A--G-------------------------------G----C---C--G-G--G-------------------------
HPBADW3     ------A-------G-ACT-----A----G-----------------GG-AT----T--A--C--T--G--AC-G--------------A--------C-C--A--T---C----GC--------------------
HPBADW2     ------A-------G-ACT-----A----G-----------------GG-AT----T--A--C--T--G--AC-G--------------A--------C-C--A--T---C----GC--------------------   58, 5
HPBADW1     ------A-------G-AC---C--A----G-------------------G-AT-----------C--T--G--AC-G---------------------C-C--AT-T---C--G--GC------------------
HPBADW2     ------A-------G-AC------A----G-------------------G--T-----------C--T--G--AC-G------------------C--C-C--A--T---C--G--GC------------------
HPBCGADR    -----T-GT-------AG--A--T----------C---------------G--TA-C----A------------C-----------------------------A--C---C--G--GCA-----------------
HBVADRM     -----T-GT-------AG--A--T----------C---------------G--TA-C----A------------C-----------------------------A--C---C--G--GCA-----------------   55
HPBADRA     -----T----------AG--A-T-A---------C-----AC--------G--T-------A------------C-----------------------------A------C--G--GCG----------TT--------
HPBCG       -----T----------AC-GA-T-A---------C-----AC--------G--T-------A------------C-------------------------C-----A------C--G--GC---------------------
HEHBVAYR    -----T----------AG--A--T----------C---------T--T---G--T-------A-------A--CC------------------------------A---C--C--G--GCA-----------------
HBVADR      -----T--A-------AG--A--T----------C---------------G--T-------A------------C-----------------------------A------C--G--GCA-----------------
HBVADR4     -----T--T-------AG--A--T----------C---------------G--T-------A------------C--------------G------------G-------A--C---C--GT-GCA-----------
HPBADR1C    -----T----------AG--A--T----------C---------------G--T-------A------------C-----------------------------A------C--GT-GCA-----------------
HPBADRC     -----T--T-------AG--A--T----------C---------T-----G--T----T--A------------C-----------------------------A------C--G--GCG----------------T------
HBVPREX     -----T----------AG--A--G----------C---------------G--T-------A------------CC---------------------------C------A------C--G--GCG----------T----------
HPBETNC     -----T--T-------AG-----T--------------------------G--T-------A------------C-----------------------------A------C--G--GCG----------------T------
HHVBC       -----T----------AG--A--T----------C---------------G--T-------A------------C------------------C--------------A------C--G--GC-------T---------------
HHVCCHA     -----T--A-------AG--A--T----------C---------------G--T-------A--------A---C------------------A--A-------------A---C--C--G--GC------------------------
HBVAYWMCG   ------AC--------AC------A---------CT-------------TGG-A--------A--G-----A--CC--------------------------C-ACAG--C---C--G---A---G-----------T------
HBVAYWC     ------AC--------AC------A---------CT-------------TGG-TT-------A--G-----A--CC--------------------------C-ACAA--TT--C--G---A---G------------------
HBVAYWCI    ------AC--------AC------A---AT----CT-------------TGG-A--------A--G-----A--CC--------------------------C-ACA---C---C--G---A---G-----------T------
HBVAYWE     ------AC--------AC------A---------CT-------------TGG-TT-------A--G-----A---C--------------------------C-ACAT--C---C--G---A---G-----------T------
HBVDNA      ------AC--------AC------A---------CT-------------TGG-TT-------A--G-----A--CC--------------------------C-ACAA--TT--C--G---A---G------------------   56
HPBHBVAA    ------TC--------AC------A---------CT-------------TGG-AT-------A--G-----A--CC--------------------------C-ACAA--CT--C--G---A---G------------------
XXHEPAV     ------AC--------AC------A---------CT-------------TGG-AT-------A--G-----A--CC--------------------------C-AGAA--GT--C--G---A---G-----------T------
HBVORFS     ------AC--------AC------A---------CT-------------TGG-AT-------A--------A--CC--------------------------A-ACAA--CT--C--G---A---G--------A--T------
HHVBE4      --A---------A-G-A-------A-----------------C-------GG--T----T--C--------A--CC-------------------A----GC-A-AA---T--C--G--GAC--G-------------------
HHVBBAS     --A---------A---A-------A-------------------------TG--T----T--C--------A--CC-------------------A----GC-A-AA---T--C--G--GA---G-------------------
HHVBF       ------AGT------ATG--A---A----------------GC-A---T---G--T----A--C--------T--CC-GC---------------A----TG-T--A--A--CT--C--G--GA---G--------T---------
HHVBFFOU    ------ACT------ATG--A---A----------------GT-A---T---G--T----A--C--------T--CC-G----------------A--A--TG-TC-A--A--CT--C--G--GA---G------------------
HBVADW4A    ------AGT------ATG--A---A----------------GC-A---T---G--T----A--C--------T--CC-GC-------------A--A--A--TG-G--A--A--CT--C--G--GA---G--------T---------   65
```

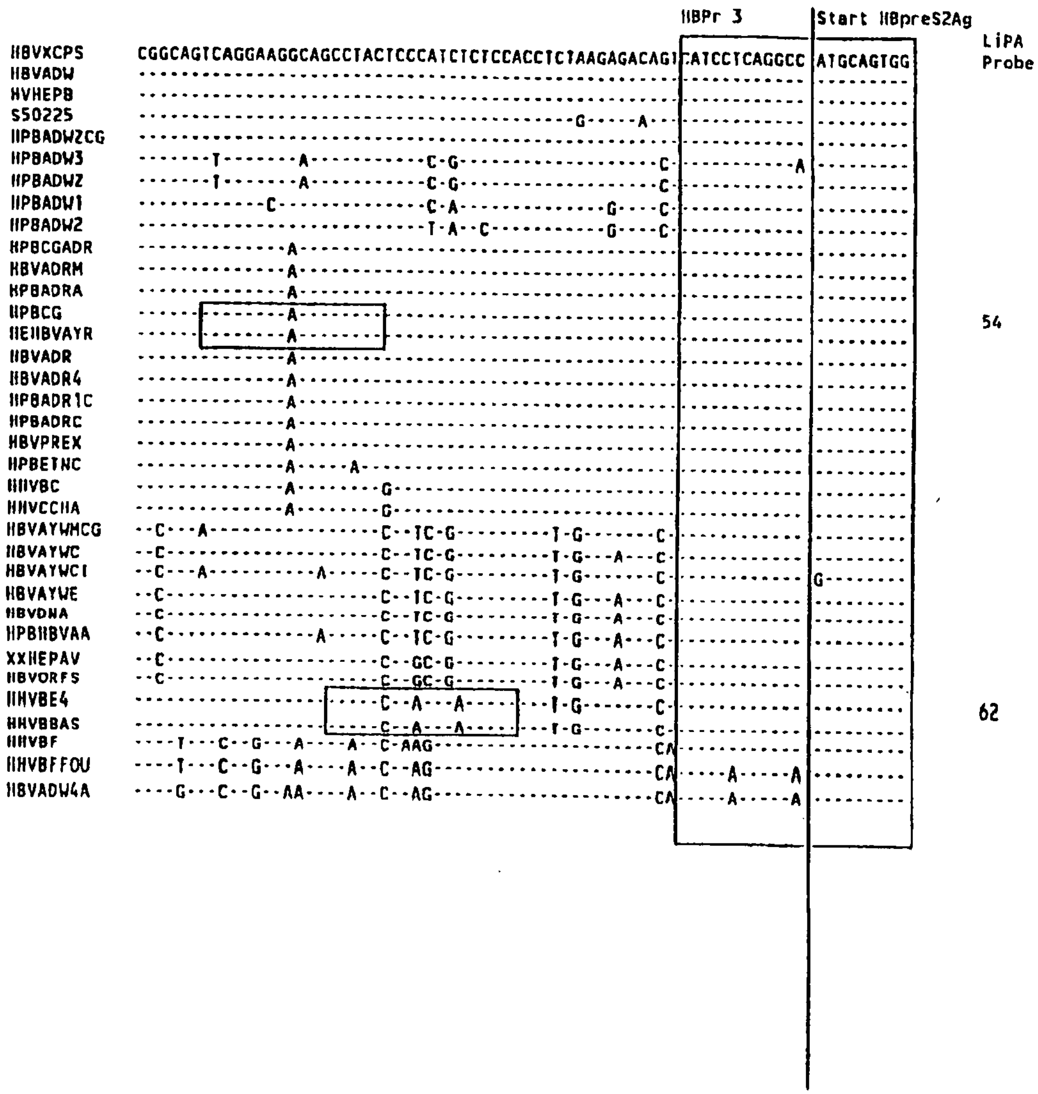

HBPr 3
Start HBpreS2Ag
LiPA
Probe
CGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCACCTCTAAGAGACAGT
ATCCTCAGGCC
ATGCAGTGG
54
62
HBVXCPS
HBVADW
HVHEPB
S50225
HPBADW2CG
HPBADW3
HPBADW2
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWCI
HBVAYWE
HBVDNA
HPBHBVAA
XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A

Figure 2: LiPA HBV design				
			HBPr	
LiPA line	Region	Purpose	Probe number/SEQ ID NO	sequence
0		Pencil line		
1		biotinylated DNA		
2	PreS1	ampl. contr.	33	CTGAGGGCTCCACCCCA
3	PreS1	Genotype A	22	AACCTCGCAAAGGCAT
4	PreS1	Genotype A	50	CCCAGAGGGTTGGGAAC
	PreS1	Genotype A	15	GCCAGCAGCCAACCAG
5	PreS1	Genotype B	57	CTGCATTCAAAGCCAACT
	PreS1	Genotype B	58	CCCCATGGGGGACTGTTG
6	PreS1	Genotype B	59	CATACTCACAACTGTGCCA
7	PreS1	Genotype C	55	TTCAACCCCAACAAGGATC
8	PreS1	Genotype C	54	TCAGGAAGACAGCCTAC
9	PreS1	Genotype D	92	TTCTGCCCCATGCTGTA
10	PreS1	Genotype D	56	AATGCTCCAGCTCCTAC
11	PreS1	Genotype D	73	TTCCACCAGCAATCCTC
12	PreS1	Genotype E	60	GGGCTTTCTTGGACGGTCC
	PreS1	Genotype E	61	CTCTCGAATGGGGGAAGA
	PreS1	Genotype E	62	CCTACCCCAATCACTCCA
13	PreS1	Genotype F	63	AGCACCTCTCTCAACGACA
14	PreS1	Genotype F	64	GCAAATTCCAGCAGTCCCG
	PreS1	Genotype F	65	GCCAATGGCAAACAAGGTA
15	preCore	promotor	88	TAGGTTAAAGGTCTTTGT
16	preCore	promotor	89	TAGGTTAATGATCTTTGT
17	preCore	scan codon -2 to +3	12	AAGTTGCATGGTGCTG
18	preCore	scan codon 1 to 5	34	ATGCAACTTTTTCACC
19	preCore	scan codon 5 to 9	13	CACCTCTGCCTAATCAT
20	preCore	scan codon 12 to 17	45	TGTACATGTCCCACTGTT
21	preCore	scan codon 12 to 17	46	TGTTCATGTCCTACTGTT
22	preCore	scan codon 16 to 20	47	ACTGTTCAAGCCTCCAAG
23	preCore	scan codon 19 to 23	48	GGCACAGCTTGGAGGCTT
24	preCore	scan codon 23 to 27	49	AAAGCCACCCAAGGCACA
25	preCore	codon 28 wt	9	TGGCTTTGGGGCATGG
26	preCore	codon 28 mt	10	TGGCTTTAGGGCATGG
27	preCore	codon 28+29 mt	42	TGGCTTTAGGACATGGA

Figure 3

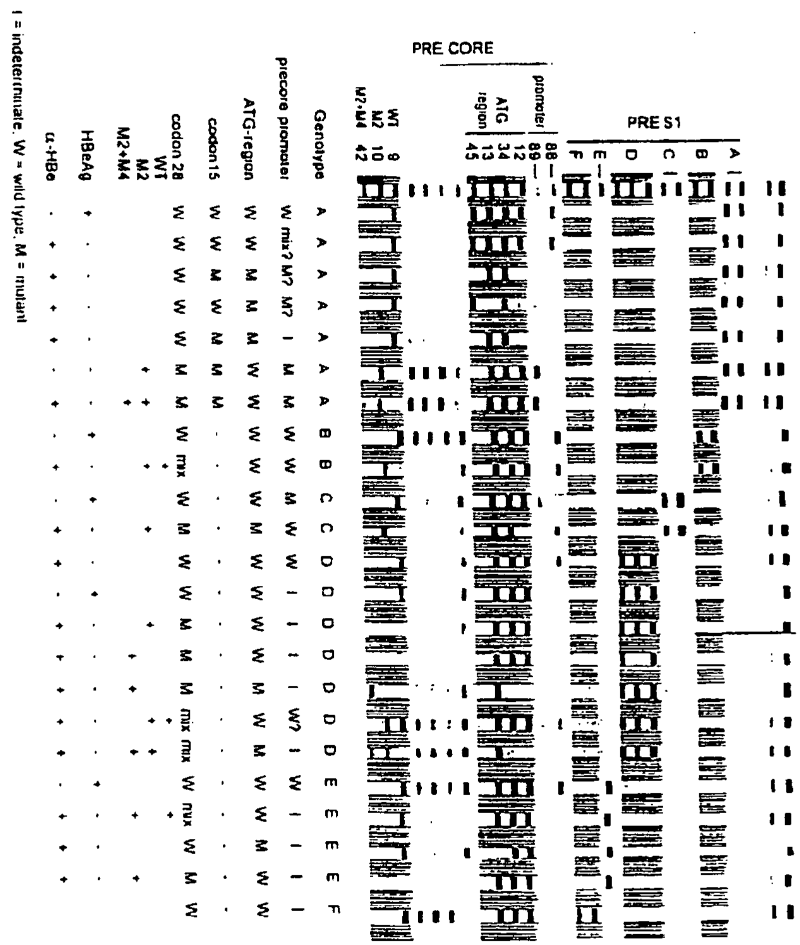

PRE CORE
PRE S1
promoter
ATG region
Genotype
precore promoter
ATG-region
codon15
codon 28
WT
M2
M2+M4
HBeAg
α-HBe
I = indeterminate, W = wild type, M = mutant

Figure 4

Genotyping in HBsAg

Genotype	Oligo	Sequence
A	HBPr 193	GGA TCA ACA ACA ACC AGT
	HBPr 140	CT CAA GGC AAC TCT ATG GG
	HBPr 77	CTA CGG ATG GAA ATT GC
B	HBPr 78	TAC GGA CGG AAA CTG C
C	HBPr 153	CT CTA CTT CCA GGA ACA G
	HBPr 154	C TGC ACG ATT CCT GCT
	HBPr 204	CT TTC GCA AGA TTC CTA TGG G
D	HBPr 165	AC TCT ATG TAT CCC TCC T
	HBPr 208	GC TGT ACC AAA CCT TCG GAT
E	HBPr 172	G GGA CCC TGC CGA AC
	HBPr 213	AG TGG TTC GCC GGG CTG G
F	HBPr 216	CA GGA TCC ACG ACC ACC AGG
	HBPr 219	GC TGT TCC AAA CCC TCG GAG
	HBPr 186	G CCA AAT CTG TGC AGC
A/B	HBPr 148	CT TTC GCA AAA TAC CTA TG
C/D/E	HBPr 80	CTT CGG ACG GAA ATT GC
E/F	HBPr 177	ATG TTG CTG TTC AAA ACC TG

Drug resistance in RT pol gene

Genotype	Oligo	Sequence	
A	HBPr 115	TCA GCT ATA TGG ATG ATG	wild type
	HBPr 116	TTC AGC TAT GTG GAT GAT	mutant
D	HBPr 127	TC AGT TAT ATG GAT GAT G	wild type
	HBPr 132	T TTC AGT TAT GTG GAT GAT	mutant

PreCore region

Genotype	Oligo	Sequence	
	HBPr 88	TAG GTT AAA GGT CTT TGT	promoter wild type
	HBPr 89	TAG GTT AAT GAT CTT TGT	promoter mutant
	HBPr 119	TGG CTT TGG GGC ATG	wild type codon 28
	HBPr 10	TGG CTT TAG GGC ATG G	mutant M2 codon 28
	HBPr 122	TGG CTT TGG GAC ATG G	mutant M4 codon 29
	HBPr 42	TGG CTT TAG GAC ATG GA	mutant M2/M4 codo

Figure 5

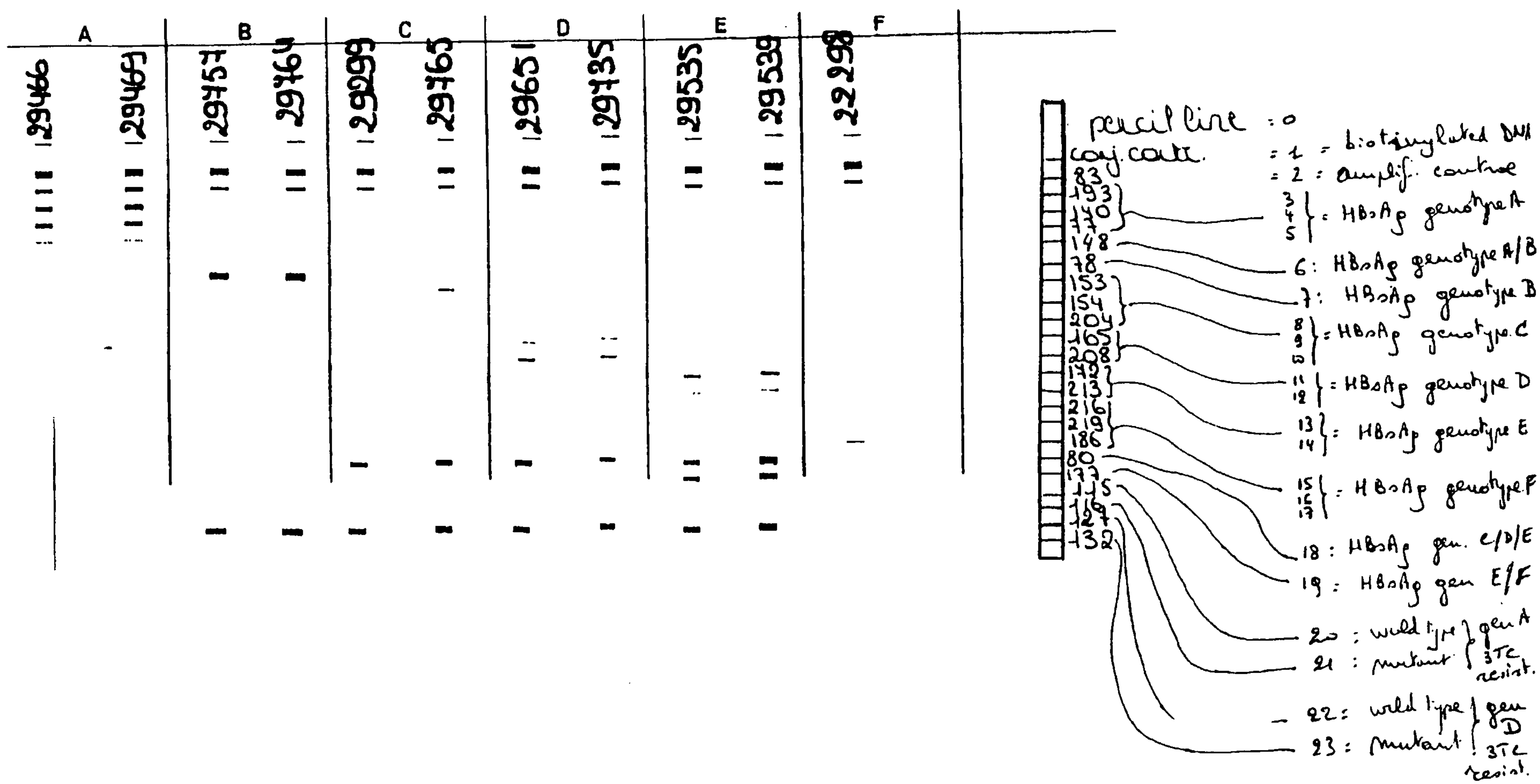
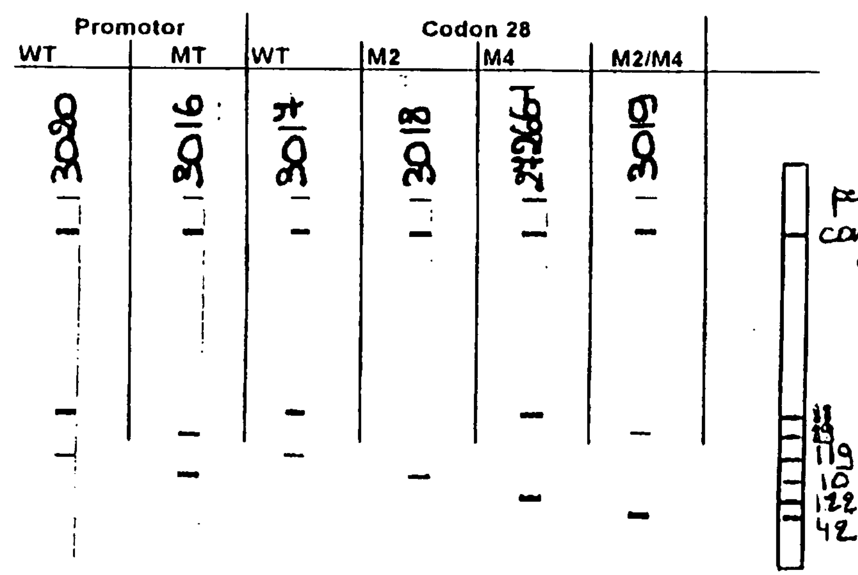

A
B
C
D
E
F
29466
29469
29457
29764
29299
29765
29651
29735
29535
29539
22298
pencil line : 0
conj. contr.
83
193
170
148
78
153
154
204
165
208
172
213
216
219
186
80
173
115
129
127
132
: 1 = biotinylated DNA
: 2 : amplif. control
3 4 5 } : HBsAg genotype A
6 : HBsAg genotype A/B
7 : HBsAg genotype B
8 9 10 } : HBsAg genotype C
11 12 } : HBsAg genotype D
13 14 } : HBsAg genotype E
15 16 17 } : HBsAg genotype F
18 : HBsAg gen. C/D/E
19 : HBsAg gen E/F
20 : wild type
21 : mutant
gen A 3TC resist.
22 : wild type
23 : mutant
gen D 3TC resist.

Figure 6



LiPA line

0 : Pencil line

1 : biotinylated DNA

2 — : precore promoter wildty

3 — : precore promoter mut

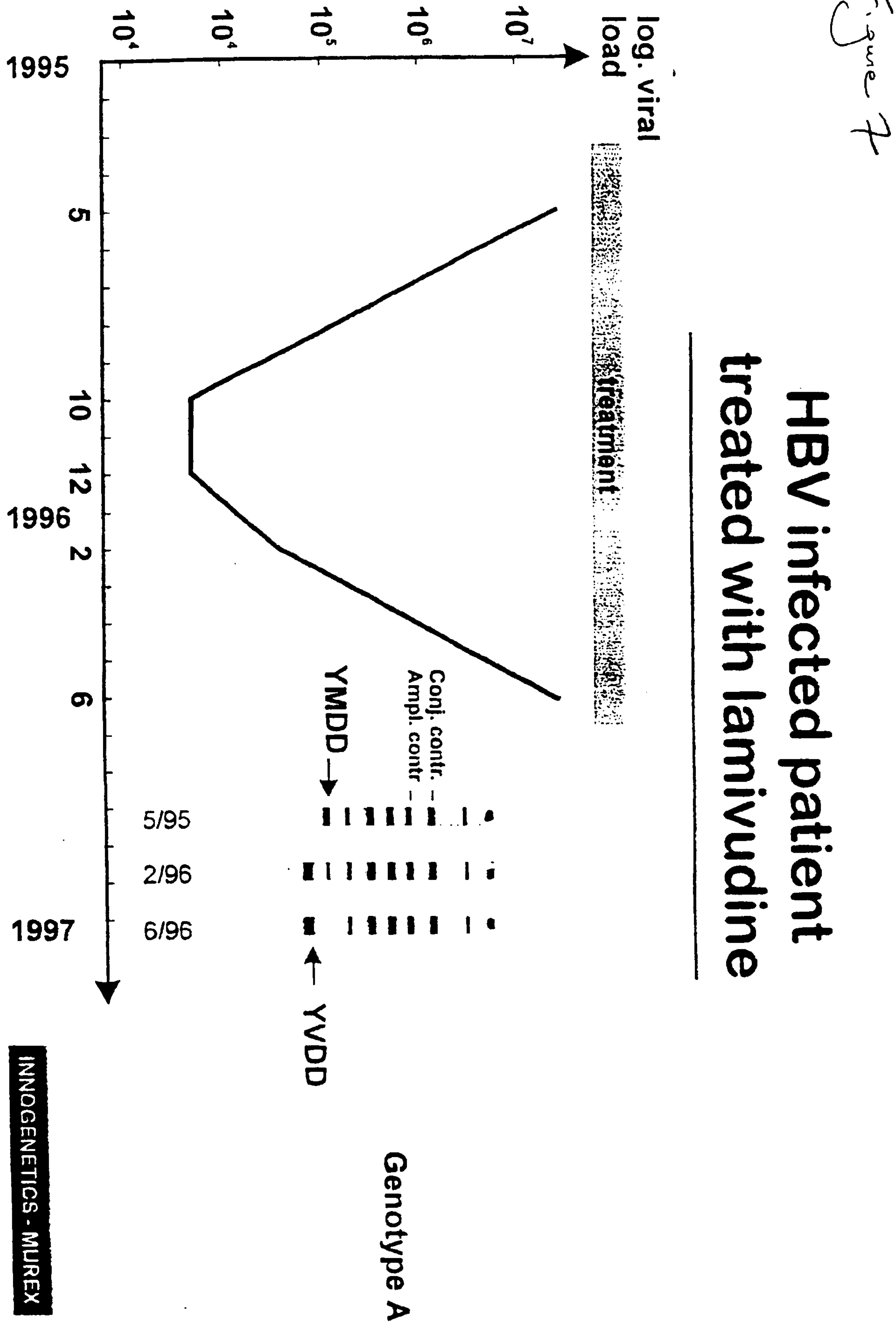
4 — : precore codon 28 wt

5 — : precore codon 28 mut

6 — : precore codon 29 mut

7 — : precore codons 28/29 m

Figure 7


HBV infected patient
treated with lamivudine
log. viral
load
10^7
10^6
10^5
10^4
10^4
1995
5
10
12
1996
2
6
1997
treatment
Conj. contr.
Ampl. contr
YMDD
YVDD
5/95
2/96
6/96
Genotype A
INNOGENETICS - MUREX